Adrenal Rest Tumours in Congenital Adrenal Hyperplasia

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List of abbreviations

CAH	Congenital adrenal hyperplasia
SW	Salt wasting
SV	Simple virilising
LO	Late onset
TART	Testicular adrenal rest tumours
ACTH	Adrenocorticotropic hormone
AII	Angiotensin II
17OHP	17-hydroxyprogesterone
A	Androstenedione
21DF	21-deoxycortisol
LH	Luteinizing hormone
FSH	Follicle stimulating hormone
DXM	Dexamethasone
CYP	Cytochrome P450

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Chapter 1

General introduction and outline of the thesis

Chapter 1.1

From gene to disease:
Congenital Adrenal Hyperplasia and the CYP21A2 gene

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Van gen naar ziekte: het adrenogenitaal syndroom en het CYP21A2 gen

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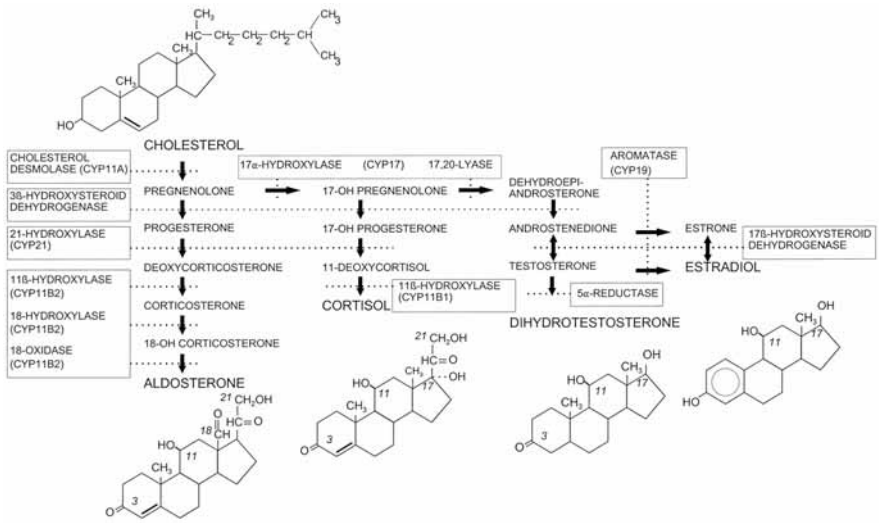
Abstract

Congenital adrenal hyperplasia (CAH) is a disorder of adrenal steroid synthesis. In more than 90% of cases CAH is caused by CYP21 (21-hydroxylase) deficiency leading to impaired cortisol and aldosterone synthesis and an increase in ACTH secretion. Chronically elevated blood ACTH concentrations result in stimulation of the adrenal gland and overproduction of androgens with prenatal virilisation of female external genitalia. The CYP21 enzyme consists of 495 amino acids and is encoded by the CYP21A2 gene located on chromosome 6p21.3 close to a 98% homologous pseudogene (CYP21p) The pseudogene contains several inactivating mutations that may be transferred to the active CYP21A2 gene by gene conversion (more than 60% of the affected alleles) or gene deletion (30% of the affected alleles). The severity of the disease depends on the degree of CYP21 deficiency. The diagnosis can be made by measuring levels of 17-hydroxyprogesterone and androstenedione in serum. In 2000 neonatal CAH screening has been introduced in the Netherlands to detect children with CAH at an early age.

Clinical aspects of congenital adrenal hyperplasia

Congenital adrenal hyperplasia (CAH) is one of the most common inherited autosomal recessive disorders and is caused by deficiency of one of the six enzymes involved in the synthesis of cortisol and aldosterone (figure 1).

Figure 1. *Steroid synthesis in the adrenal cortex. Cortisol, aldosterone and androgens are synthesized via six enzymatic steps from the precursor cholesterol. Deficiency of one of the enzymes involved in steroid synthesis results in impaired production of adrenal steroids.*



In more than 90% of cases CAH is caused by CYP21 (21-hydoxylase) deficiency leading to an impaired production of cortisol and aldosterone by the adrenal cortex (1-3). Consequently, the secretion of ACTH is increased due to the loss of negative feedback on the pituitary gland leading to hyperplasia of the adrenal gland. As a result of increased pituitary ACTH production, steroids before the enzymatic block, 17-hydroxyprogersterone (17OHP) and androstenedione (A), are produced in excess. Disorders of adrenal steroid genesis can also be caused by deficiencies of 11-hydroxylase, 3β hydroxysteroid dehydrogenase (HSD), 17 α-hydroxylase, steroidogenic acute regulatory protein or P450 oxidoreductase (POR). This thesis will be limited to CAH due to CYP21 deficiency.

The clinical picture of CAH depends on the severity of the enzymatic bloc (1). The classic salt wasting (SW) type is caused by complete CYP21 deficiency leading to prenatal virilisation of the external genitalia in girls due to prenatal exposure of adrenal androgens and

to the development of Addisonian crisis in girls and boys due to impaired cortisol and aldosterone synthesis. Patients with the classic simple virilising (SV) type have a residual enzyme activity of 1 – 2% that is generally enough for sufficient aldosterone production to prevent salt wasting. However, these children do not synthesize enough cortisol and androgen excess leads to prenatal virilisation of the external genitalia in girls. In the non-classic late onset (LO) type of CAH the residual enzyme activity of CYP21 is usually 30 – 50%. These patients produce normal amounts of cortisol and aldosterone but produce elevated amounts of adrenal androgens leading to a clinical picture of pseudo puberty in childhood or acne, hirsutism or menstrual disorders in adulthood (4). In the classic type of CAH medullar adrenal function may also be impaired resulting in insufficient synthesis of epinephrine and a higher risk of hypoglycemia in stress situations in children (5-7).

The aim of treatment in CAH patients is substitution of glucocorticoids and suppression of the adrenal production of androgens (8). In children the preferred drug is hydrocortisone. Patients with additional aldosterone deficiency also require supplemental mineralocorticoids (fludrocortisone) that are usually given once or twice a day. In the first year of life most patients need additional sodium suppletion because the sodium content of breast milk and infant formulas is generally insufficient for these patients to compensate for salt wasting.
It is important to use a dose of glucocorticoids that also adequately suppresses adrenal androgens. Insufficient therapy with glucocorticoids causes elevated adrenal androgen levels leading to increased growth velocity, signs of pseudo puberty and a reduced final height (9-11). On the other hand overtreatment can result in Cushing's syndrome with growth retardation, obesity, hypertension and also reduced final height (12-14). Therefore, in CAH children therapy has to be monitored each 3 months by evaluating clinical symptoms, anthropometrical measurements (height and weight) and determination of 17OHP and A concentrations in serum or saliva or their metabolites in urine.

In adult CAH patients poor hormonal control can interfere with gonadal function. Adult females can present with irregular menses, amenorrhea and subfertility (15-17). In male patients with CAH testicular adrenal rest tumours (TART) can occur which can also interfere with gonadal function (18-19). Intensifying glucocorticoid therapy may lead to shrinkage of the tumours and improvement of gonadal function (19,20). When intensifying glucocorticoid therapy does not lead to tumour regression within several weeks or the patient develops serious side effects, surgical treatment has to be considered to prevent testicular damage in

longstanding TART (21-23). Furthermore, suppressed gonadotropins due to elevated adrenal androgens can also lead to gonadal dysfunction and infertility (24). Fertility in adult CAH patients is discussed in detail in the following chapter (chapter 1.2).

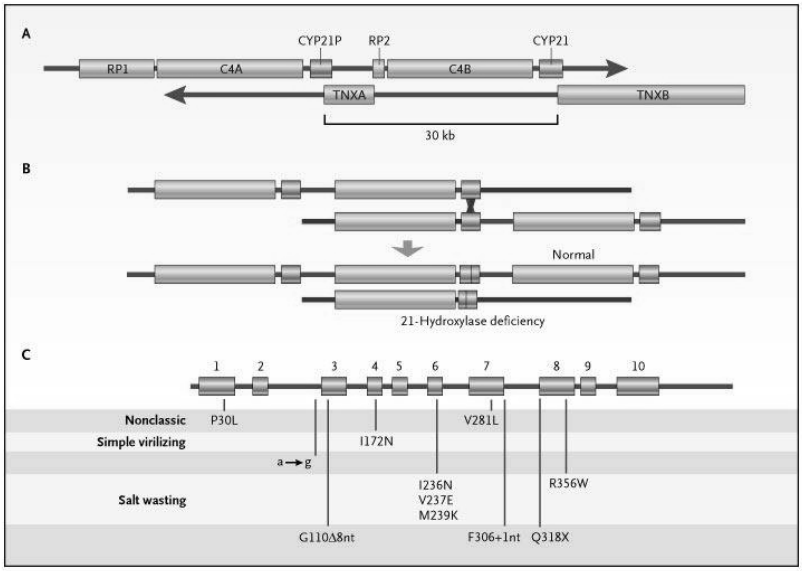
Girls with the classic type of CAH are exposed to high levels of adrenal androgens from approximately the seventh week of gestation resulting in virilisation of the external genitalia. However, not all classic CAH females, even siblings, develop the same degree of genital ambiguity. This may be explained by differences in androgen levels, efficiency of conversion of precursors to more potent androgens and variation in androgen receptor expression. The uterus and ovaries are normally formed and internal Wolffian structures such as prostate and spermatic ducts are not present (25-26). Surgical corrections of the external genitalia are mostly performed in the first year of life (27).

The gene

The CYP21A2 gene is located on chromosome 6p21.3 within the HLA major histocompatibility complex. The gene consists of 10 exons and is 98% identical to an inactive pseudogene (CYP21p) that is located close to the CYP21A2 gene (28,29) (figure 2).

The pseudogene contains several mutations that are responsible for complete inactivation of its gene product. Approximately 60% of the mutations causing CYP21 deficiency arise from mutations found in that pseudogene, that are transferred to the CYP21A2 gene by gene transfer (30). About 30% of the mutations are caused by unequal cross-over during meiosis resulting in complete deletion of the gene (1,29). 1–2% of the mutations are spontaneous mutations not present in one of the parents (31). Until now approximately 60 mutations causing CYP21 deficiency have been described (32 (www.cypalleles.ki.cyp21.htm). The most common mutations in CYP21 are a deletion (20% of the affected mutations) and an A→ G substitution before the end of intron 2 that results in aberrant splicing of pre-mRNA (approximately 20% of the affected alleles) (33-34). The phenotype depends on the degree of the enzyme deficiency with an overall genotype-phenotype correlation of 80% (35-39). Patients who are compound heterozygote (i.e. two different mutations in two alleles) generally have the phenotype of the less severe mutation. Heterozygous carriers have an exaggerated 17OHP concentration in response to ACTH stimulation suggesting an impairment of enzyme activity in carriers (40-41). However, there is still disagreement whether heterozygous carriers are at risk for the development of hyperandrogenism (42-44).

Figure 2. *Schematic overview of the CYP21A2 gene. (adapted from N Engl J Med 2003; 349:776-788).*



A. The CYP21A2 gene is located on chromosome 6p21.3. near the pseudogene CYP21p, which contains several mutations that completely inactivate this gene.
B. Unequal cross-over leading to complete deletion of the CYP21A2 gene.
C. The most common mutations causing CYP21A2 deficiency with associated phenotype.

The protein

The Cyp21A2 gene encodes for the enzyme CYP21 (also named P450c21) consisting of 495 amino acids with a molecular mass of approximately 52 kDa. CYP21 belongs to the family of cytochrome P450 (CYP) enzymes that are involved in a variety of biochemical reactions (1). Six CYP enzymes are involved in the steroid synthesis. The enzyme CYP21 is located in the endoplasmatic reticulum and is responsible for hydroxylation of the steroid precursors on the C_{21} position. Therefore, the CYP21 enzyme is also called 21-hydroxylase. CYP21 converts 10 17OHP to 11-deoxycortisol and progesterone to deoxycorticosterone (DOC). A homozygous deletion of the CYP21A2 gene results in complete loss of function of the CYP21 enzyme with severe salt wasting. Several point mutations result in an enzyme with an activity of approximately 1% (for example the I172N mutation) that allows sufficient aldosterone synthesis to prevent salt wasting. Other mutations result in an enzyme activity of 20 – 50% of the normal activity. These patients have sufficient cortisol and aldosterone production but have increased adrenal androgens (4).

The cell

CYP21 is exclusively present in the zonae fasciculata and glomerulosa of the adrenal cortex (45). The first step in the steroid synthesis is transfer of cholesterol to the mitochondria. The mitochondrial enzyme CYP11A (p450cholesterol desmolase) catalyzes the conversion from cholesterol to pregnenolone. Pregnenolone is the common precursor for all steroids in the adrenal cortex and is transferred to the endoplasmatic reticulum where further conversion is catalyzed by CYP17 (17-hydroxylase) and CYP21. The final step in the cortisol and aldosterone synthesis takes place in the mitochondria. The most important factor in the regulation of CYP21A2 gene expression in the cell is ACTH. ACTH binds to a specific G protein-coupled receptor resulting in an increased intracellular cAMP concentration. This leads to an increase of cholesterol transfer across the mitochondrial membrane and activation of CYP21A2 expression mainly through increased transcription and resulting in increased cortisol production (46). Cortisol has a negative feed back on the hypothalamus and pituitary gland thereby decreasing the ACTH secretion.

The population

The incidence of the classic CYP21 deficiency in the Netherlands is 1: 12 000. The incidence of the late onset type of CAH is much higher with an incidence of approximately 1:1700 and may be underestimated because the diagnosis can be easily missed, especially in males, in whom the androgen excess is not clinically visible. In certain populations such as in Eastern European countries the late onset type of CAH is much more frequent (up to 1-2%). The overall carrier frequency of CAH mutations is 1: 50 (47).

Diagnosis

The diagnosis CAH due to CYP21 deficiency can be made by detection of elevated serum levels of the steroid precursors 17OHP and A. 17OHP and A levels show a diurnal rhythm with a highest concentration early in the morning. Therefore, it is necessary to measure 17OHP and A in the morning for diagnosis and monitoring to detect slightly elevated 17OHP and A levels. ACTH is not a specific marker of the disease and is elevated in all types of

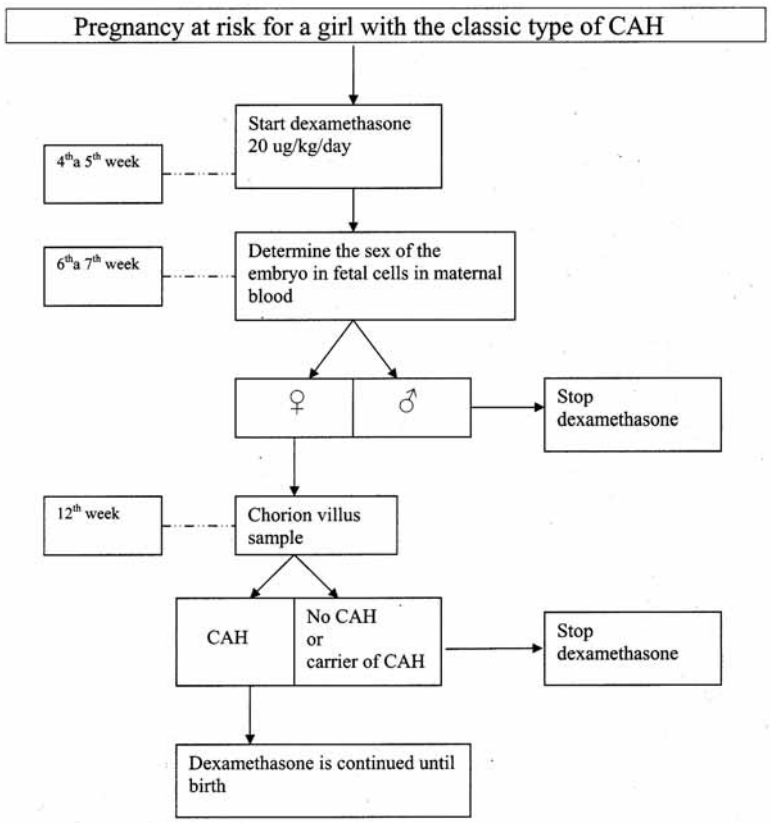
primary adrenal insufficiency. When there is doubt about the diagnosis an adrenal stimulation test with cosyntropin (synacthen^{R}) can be performed (1,2).
Because of the possibility of cross reactivity with other steroids within the hormone assay it is necessary to extract the steroids from blood and to separate the steroids with chromatography before measuring the steroid concentrations. 17OHP and androstenedione concentrations can also be determined in salivary samplings taken just before ingestion of the glucocorticoid medication (48). The main urinary metabolite of 17OHP is pregnantriol (P3), which can be detected in urine collected for 24 hours ("steroid profile"). The renin level in serum can be measured to detect aldosterone deficiency in the case of clinical salt wasting.
In 2000 a neonatal CAH screening program has been introduced in the Netherlands. In a blood sample from the heel puncture the level of 17OHP is determined (49). Aim of the neonatal screening program is to detect asymptomatic neonates, especially boys, at an early stage to prevent severe salt wasting and Addisonian crisis. In the Netherlands 15 – 20 patients are detected by the screening program every year. In the case of elevated 17OHP levels (i.e. a positive screening result) the patient is transferred to a pediatric endocrine centre for additional tests as described above. The diagnosis CAH due to CYP21A2 deficiency can be confirmed by mutation analysis.

Prenatal diagnosis and treatment

In pregnancies at risk for a girl with the classic type of CAH (i.e. SW and SV) and virilisation in utero, suppression of adrenal androgen production can be achieved by administering dexamethasone (DXM) to the mother (50). Virilisation of the external genitalia starts at the 6th week of gestation. Therefore, DXM has to be administered as soon as possible. The dose is 20 μg/kg/day in three divided doses with a maximum to 1.5 mg daily. In the 7th week of gestation blood samples of the parents are taken to determine the sex of the embryo in fetal cells in maternal blood. In the case of a male sex DXM treatment is discontinued. In the case of a female karyotype DXM treatment is continued and a chorion villus sample is taken around the 12th week of gestation for molecular analysis of the CYP21A2 gene. If the female fetus is affected, the glucocorticoid treatment is continued until birth. Approximately 70 – 80% of prenatally treated CAH females are born with normal or only slightly virilized external genitalia. Adverse events of the mother during DXM treatment are excessive weight gain, striae and hypertension during pregnancy.

These side effects commonly resolve with discontinuation of DXM treatment. Adverse effects for the child are difficult to determine and long-term neuropsychological studies are following. In a recent paper it is suggested that prenatal DXM treatment is associated with previously not described long-term effects on verbal working memory and on certain aspects of self-perception. Sporadic cases of intrauterine growth retardation, palate cleft and hydrocephalus have been described (51).

Figure 3: *Prenatal diagnosis and treatment in pregnancies at risk for a girl with the classic type of CAH.*



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Chapter 1.2

Fertility in patients with congenital adrenal hyperplasia

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Summary

Congenital adrenal hyperplasia (CAH) is generally regarded as a pediatric endocrine disease, but nowadays nearly all patients reach adulthood as a result of improved diagnosis and treatment. It is now increasingly recognized that treatment goals shift during life: one of the major treatment goals in childhood and puberty, i.e. normal growth and development, is no longer relevant after childhood, whereas other items, such as fertility and side effects of long-term glucocorticoid treatment, become more important in adulthood. This paper focuses on fertility in male and female adult CAH patients.

In CAH males' fertility rate is reduced compared with the normal population, the most frequent cause being testicular adrenal rest tumours. Development and growth of these tumours is assumed to be ACTH dependent and undertreatment may play an important role. If intensifying of glucocorticoid treatment does not lead to tumour decrease, surgical intervention may be considered, but the effect on fertility is not yet known.

In CAH females the degree of fertility depends on the phenotype of the CAH. Most fertility problems are seen in the classic salt-wasting type. Age of menarche and regularity of menstrual cycle depends on the degree of adrenal suppression. Not only adrenal androgens have to be normalized but also the levels of adrenal progestin (progesterone and 17-OH progesterone) that interfere with normal ovulatory cycles. In reverse the regularity of menstrual cycles can be considered as an important measure of therapeutic control in adolescent female CAH patients and therefore as a therapeutic goal from (peri) pubertal years on. Other factors that contribute to impaired fertility in CAH females are ovarian hyperandrogenism (polycystic ovary syndrome), ovarian adrenal rest tumours, genital surgery and psychological factors.

In conclusion subfertility in CAH can have its origin already in peripubertal years and therefore has to be of interest for the pediatric endocrinologist.

Introduction

Congenital adrenal hyperplasia (CAH) is a disorder of adrenal steroid synthesis. In 95% of cases, it is caused by 21-hydroxylase deficiency, leading to cortisol deficiency and (in most cases) aldosterone deficiency. The compensatory increase in ACTH secretion by the pituitary gland leads to stimulation of the adrenals and, consequently, overproduction of androgens. The phenotype of 21-hydroxylase deficiency depends on the degree of enzyme deficiency. Complete 21-hydroxylase deficiency leads to the classic salt-wasting form with congenital virilisation in females. Less severe 21-hydroxylase deficiency results in the classic simple virilising form without aldosterone deficiency. Patients with the mildest form, the late onset form of CAH, present with symptoms caused by androgen excess only: pseudo precocious puberty, hirsutism, menstrual irregularities and infertility (1). Treatment of 21-hydroxylase deficiency consists of glucocorticoid suppletion and in case of aldosterone deficiency also mineralocorticoid suppletion.

In the past, CAH was generally regarded as a pediatric endocrine disease, but nowadays nearly all patients reach adulthood as a result of improved diagnosis and treatment. Thus, the spectrum of CAH as a lifelong chronic disease becomes gradually clear, and it is increasingly recognized that treatment goals shift during life: the major treatment goals in childhood and puberty, i.e. normal growth and development are no longer relevant after childhood, whereas other items, such as fertility and side effects of long-term glucocorticoid treatment, become more important in adulthood. In this paper we focus on fertility in adult male and female CAH patients.

Fertility in male patients with congenital adrenal hyperplasia

There is evidence that fertility is impaired in male CAH patients. Jääskeläinen et al. found a significantly lower child rate (0,07) in 16 male CAH patients compared with an age matched Finnish male control group (0,34) (2). Other studies report child rate only as additional information in selected patient populations(3-5). An alternative method to investigate fertility in men is semen analysis. Wuesthof et al. reported normal semen analysis in only 34% of 53 male CAH patients (4). Cabrera et al. reported abnormal semen analysis in 46% of 16 investigated patients (5). In our own series only 4 of 11 adult CAH patients had a normal sperm count (6). However, not all patients are willing to collect semen for analysis.

Measurement of serum FSH and inhibin B levels is also used to assess fertility in male CAH patients. Several studies report abnormal FSH levels, indicating Sertoli cell damage (2,3,5,6). However, it should be noted that in CAH patients with primary testicular damage, serum gonadotropin levels might be suppressed due to high levels of adrenal androgens. Most of these patients also have serum testosterone levels within the normal range, which is the result of conversion of adrenal androgens to testosterone. In this situation a more reliable predictor of testicular damage is the serum inhibin B level, which reflects Sertoli cell function.
The most important cause of infertility in male patients with CAH is the presence of testicular tumours resulting in primary gonadal failure. Another important factor contributing to infertility is the suppression of the hypothalamic-pituitary-gonadal axis due to high circulating levels of androgens resulting in secondary gonadal failure.

Testicular adrenal rest tumours

Testicular tumours in male CAH patients are thought to arise from aberrant adrenal cells in the testes that are stimulated by ACTH (7). Therefore they are called testicular adrenal rest tumours. Also in other conditions with high plasma ACTH levels, such as in Nelson's syndrome or Addison's disease, testicular adrenal rest tumours have been described (8,9). However, also in well-controlled CAH patients, with normal or suppressed ACTH, testicular tumours are found (5,6). The reported prevalence of testicular adrenal rest tumours in males varies between 0 and 94%, dependent on the selection of the patients and the method of detection (2,3,5,6,10,11) . In our own series, in 16 of the 17 patients (age 16-40 years) one or more testicular tumours were found. Ultrasound seems to be the best method for detection and follow up, especially in case of small non-palpable tumours (12). Testicular tumours have also been reported in patients under the age of 16 (10,13,14). We now routinely follow our male children with CAH (n = 25;age 6 – 19 years) with ultrasound and detected 7 boys with small, mostly non-palpable tumours, the youngest patient being 7 years old (unpublished data). The testicular tumours, which are always located in the mediastinum testis, can lead to obstruction of the vascular supply and compression and atrophy of the seminiferous tubules. In addition to these mechanical effects, steroids produced by the tumours could be toxic to testicular tissue (paracrine effect) therefore contributing to testicular failure (15). These two factors result in primary gonadal failure with elevated gonadotropin levels and low inhibin B levels. Therefore treatment or prevention of the development of testicular tumours is an important goal. Poor hormonal control with inadequate suppression of ACTH may be a main

factor in the etiology of testicular tumours (16-18). By increasing the glucocorticoid dose, ACTH secretion is suppressed and the adrenal rest tissue is no longer stimulated, which may lead to testicular tumour shrinkage. The need for tumour shrinkage and the side effects of increasing glucocorticoid therapy must be carefully balanced, especially in asymptomatic cases (19). The effect of medical treatment is described mostly in case reports or in small patient groups and the results vary dependent on patient selection, type of CAH and treatment choice (14,20-24). Rutgers et al. reported tumour shrinkage in 75% of 16 patients after increasing the glucocorticoid dose (21). Walker et al. reviewed 75 cases of testicular tumours in CAH and stated that the majority of the masses regressed with an increase in glucocorticoid dose (22). Rich et al. reported testicular tumours in 3 young patients (5,15 and 17 years old). Intensifying of glucocorticoid therapy resulted in normalizing of the elevated steroid levels in all cases. However in no case the tumours resolved, in only one case there was partial regression (14). In our own study population we also found unpredictable results (23): tumour decrease was found in 6 of the 15 male CAH patients after intensifying of glucocorticoid therapy, but tumour decrease was also seen in 1 patient with undertreatment. Tumour increase was also seen in patients with adequate treatment. Use of dexamethasone instead of hydrocortisone in the night resulted in better adrenal suppression in only one of our patients. Therefore general guidelines for using glucocorticoid strategies can not been given and the decision for dose and timing of the glucocorticoid treatment has to be made individually in all patients, with special attention to side effects.

If tumour size does not decrease with increasing glucocorticoid therapy or if there is persistent azoospermia despite tumour shrinkage, surgical intervention may be considered (22,25). In the past orchiectomy was performed. However the malignant potential of these tumours is negligible (26). Walker performed testes sparing surgery in 3 patients with CAH (22). Postoperatively there was good vascular flow and no recurrence of the tumour. However investigations to assess fertility were not performed. Tiryaki et al. also reported 2 male CAH patients with steroid unresponsive testicular tumours who were also treated by testis sparing tumour enucleation (25). Again no data about fertility before and after operation were reported. Because fertility prognosis in CAH males with testicular tumours remains uncertain, cryopreservation of semen could be proposed to young adult male patients. In case of unwanted infertility, assisted reproduction could be considered. When there is obstructive azoospermia, testicular aspiration and intracytoplasmatic sperm injection may offer a solution (15). At least in some cases deterioration of fertility by testicular tumour growth can be

prevented by early intensifying glucocorticoid therapy. Furthermore early testis sparing surgery as soon as testicular tumours are detected has the potential to prevent damage of testicular tissue. Therefore it may be that detection of the tumours by ultrasonography in an early stage (prepubertal) is useful. However, further investigations are necessary to support this hypothesis.

Hypogonadotropic hypogonadism

In poorly controlled CAH patients the elevated ACTH levels induce high levels of androstenedione, which is partly aromatized to oestron. These high levels of androgens and oestron will suppress the hypothalamic-pituitary-gonadal axis leading to hypogonadotropic hypogonadism and small testes (17-18-27). It may be that steroids produced by testicular adrenal rest tumours contribute to the suppression of the hypothalamic-pituitary-gonadal axis. However this effect cannot be separated from the effects resulting from adrenal androgen excess.
Hypogonadotropic hypogonadism may also occur in males with previously undiagnosed late onset 21-hydroxylase deficiency. In this condition adrenal rest tumours are not a common finding. Most reports show reversible hypogonadism and improved fertility after initiating or increasing glucocorticoid therapy (28,29).

Fertility in women with congenital adrenal hyperplasia

In women with CAH due to 21-hydroxylase deficiency, fertility seems to be reduced, based on reports of decreased pregnancy rates, decreased live-birth rates and menstrual disorders (30,31).
Most reports about pregnancies in female patients with classic 21-hydroxylase deficiency are case reports. There are only a few reports providing pregnancy rates or live-birth rates in large populations of CAH patients (32-36): the live-birth rate in classic salt-wasting CAH patients is 0 to 10% (n = 64), in simple virilising patients 33 to 50% (n = 83) and in non-classic patients 63 to 90% (n = 18). In the general population or in age-matched controls, pregnancy rates or live-birth rates are 65 to 91%(32-35). Thus, compared with a non CAH female population, pregnancy and live-birth rates are severely reduced in salt-wasting CAH patients,

mildly reduced in simple virilising CAH patients, and normal in non-classic CAH patients. Pregnancy outcomes in women with classic CAH have been reviewed by Lo and Grumbach (37). They found 105 reported pregnancies in 73 CAH women (20 salt-wasting patients, 53 simple virilising patients), resulting in 74 live-born children. Of these 105 pregnancies, 11 (10%) led to spontaneous abortion and 11 (10%) were electively terminated.

Data on fertility in patients with non-classic 21-hydroxylase deficiency are predominantly derived from studies in patients in whom the diagnosis of CAH was made after they had presented with subfertility and/or other symptoms of hyperandrogenism. As a result, these fertility data represent only the symptomatic subset of the non-classic CAH population, and this introduces substantial bias. It has become clear that the prevalence of non-classic CAH is relatively high, but when the disease is mild, patients may never come to clinical presentation. In 2 reports on fertility in non-classic patients, presenting with subfertility, the corrected pregnancy rate was 93% and 100% (38,39), spontaneously or after glucocorticoid treatment (with or without clomiphene citrate). Instead of pregnancy rates and live-birth rates as direct markers of fertility, regularity of menstrual cycles can be used as an indirect marker of fertility, particularly in adolescent girls. In most reports, a normal mean age of menarche in CAH girls was observed (40-42), but these data are misleading because by definition only the patients who did experience menarche were included. In CAH women, delayed menarche can be associated with poor therapeutic control. Menstrual irregularity in women with CAH has also been associated with poor therapeutic control (41) and in non-classical CAH, menstrual irregularity is typically one of the presenting signs.

Several factors have been suggested to contribute to impaired fertility in CAH females: adrenal overproduction of androgens and progestins (17-hydroxyprogesterone and progesterone), ovarian hyperandrogenism (polycystic ovary syndrome), ovarian adrenal rest tumours, genital surgery and psychological factors such as delayed psychosexual development, reduced sexual activity and low maternal feelings.

Adrenal overproduction of androgens and progestins

Androgen overproduction by the adrenal gland can directly and indirectly affect ovarian activity. Directly, androgen excess inhibits ovarian folliculogenesis. The hypothesis, that androgen excess has a negative (direct and/or indirect) effect on ovulation, is supported by the finding, that suppression of adrenal androgen secretion by increasing the glucocorticoid dose

can restore ovulation in CAH patients. However, in some patients adequate suppression of androgen levels was not sufficient to correct menstrual abnormalities. In these patients, increased levels of progestins (progesterone and 17-hydroxyprogesterone) as a result of adrenal overproduction interfered with normal menstrual cycles(34,41,43,44).
Elevated progestin levels may cause persistent inhibition of follicular growth, inhibition of endometrial proliferation and failure of endometrial breakdown, resulting in menstrual disorders. In addition, even if regular ovulation and menstruation is achieved, elevated progesterone levels from adrenal origin can still prevent conception in CAH females, by causing involution of the endometrium and impermeability of the cervical mucus (45).
Thus, adequate suppression of adrenal androgens and progestins is needed for menarche and regular menstrual cycles. In reverse, the regularity of menstrual cycles can be considered as a measure for therapeutic control in female CAH patients and should be aimed at from pubertal years on.

Hypogonadotropic hypogonadism

Similar to in male CAH patients, oversecretion of androgens, which are mostly aromatized to oestron, can result in hypogonadotropism, contributing to anovulation or dysovulation. Adequate suppression of androgens can help to restore gonadotrophine cyclicity and therefore regular menstrual cycles. However, in contrast to the experience in male CAH patients, these findings are rare in female patients (46,47).

Polycystic ovarian syndrome (PCOS)

PCOS is characterized by ovulatory dysfunction and hyperandrogenism with irregular menstrual cycles, hirsutism and acne leading to subfertiliy. In the classic form polycystic ovaries are detected. The pathogenesis of PCOS is still uncertain but there is evidence that the androgens result from ovarian overproduction (48). Female CAH patients with poor hormonal control as well as untreated women with the non-classic form of CAH can have a similar clinical presentation including sonographic evidence of ovarian cysts resulting from adrenal hyperandrogenism. In both conditions significantly elevated levels of androgens, 17-hydroxyprogestrone and insulin insensitivity have been described (49-52). So the distinction between these 2 conditions can be difficult. Differentiation can be made upon post ACTH rise in 17OHP and molecular analysis (52,53). Because PCOS is associated with reduced fertility

(54) it is suggested that the presence of PCOS in CAH patients can be an additional factor in the mechanism of subfertility in CAH females (1,55).
Stikkelbroeck et al. investigated the prevalence of PCOS in 13 female CAH patients. Polycystic ovaries were found in 2 patients (15,4%) reflecting a prevalence corresponding to the general population (56). Therefore it is unlikely that PCOS is a frequent cause of infertility in CAH patients.

Ovarian adrenal rest tumours

In contrast to the high prevalence of testicular tumours in male CAH patients ovarian adrenal rest tumours have been described only in case reports (57-59). In our own study of 13 adult, female CAH patients no ovarian adrenal rest tumours could be detected neither by ultrasonography nor on MR imaging which suggests that ovarian adrenal tumours are rare and do not contribute to female subfertilty frequently (56). Therefore routine ovarian imaging in these patients is not indicated.

Genital surgery

Besides the endocrine factors described above, the effects of genital surgery in early life also play an important role in the impaired fertility in CAH females. Surgical reconstruction of ambiguous genitalia in 46 XX neonates consists of reduction of clitoral size, creating of labia minora and exteriorization of the vagina therefore creating a functional vagina to allow menstruation and sexual activity (60,61). The most important factors related to surgery that can interfere with sexual disturbance are loss of clitoral sensibility, intravaginal stenosis and disturbed vaginal arousal (62). In the past in female neonates with clitoromegaly, clitoridectomy was performed with loss of sensibility due to damage of neurovascular supply.
Currently the most preferred technique is clitoroplasty with excision of the erectile tissue preserving the neurovascular supply to the glans (63). However, recent data show that there is still abnormal clitoral sensation even after optimalizing of surgical techniques (63). The incidence of vaginal stenosis after surgery varies dependent on the type of operation (64,65). In the past stenosis was reported up to 77% (18). Krege et al. reported vaginal stenosis in 36% of the patients with the requirement of additional treatment (manual dilatation, secondary vaginoplasty) (66). Therefore modern surgical techniques performed by experienced surgeons can improve the functional results of the surgery.

Psychosexual factors

Several studies show gender atypical behavior in female CAH patients (67-75). It is suggested that biological and social factors contribute to the development of gender identity disorders in these patients. Pre- en postnatal exposure to androgens can cause gender atypical behavior resulting in more boyish behavior such as preference for male typical toys and admiration for male characters. Furthermore significantly less satisfaction with the female sex of assignment is reported (68). However social factors also can influence the development of gender identity such as inability of parents to accept sex assignment (67). These gender identity disorders can contribute to subfertility in female CAH patients (69). There is controversy about the rate of bisexuality or homosexuality. Other psychosocial factors can be anxiety about sexual activity and inability to achieve orgasm.

Conclusion

Congenital adrenal hyperplasia and its treatment have a considerable impact on fertility in males and females. It is important to recognize, that the majority of causes impairing fertility are founded in childhood years and therefore should be a treatment goal already in (peri) pubertal years. Therefore CAH should be regarded as a lifelong disease: the implications of CAH and its treatment reach beyond childhood.

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Chapter 1.3

Outline of the thesis

Outline of the thesis

Congenital adrenal hyperplasia (CAH) is the most common inherited disorder affecting adrenal steroid synthesis. In more than 90%, CAH is caused by CYP21 (21-hydroxylase) deficiency. The clinical presentation depends on the severity of the enzyme deficiency with in the most severe cases complete cortisol and aldosterone deficiency causing an Addisonian crisis within the first weeks of life and prenatal virilisation of the affected females. Nowadays, the diagnosis of the most severe type of CAH can be made at an early age by neonatal screening programs or by prenatal diagnosis in the case of an affected proband, thereby preventing life threatening events. Most children reach adult age without severe morbidity. However, in adult CAH patients several complications can develop and in recent years it became clear that some of them might be detected already in childhood. One of the most important and frequently detected complications in male CAH patients is the development of testicular tumours. These tumours have no malignant features. However, because of their location in the mediastinum testis they can lead to obstruction of the seminiferous tubules leading to gonadal dysfunction and infertility. It is thought that the tumours consist of adrenal-like tissue. Therefore, they are called testicular adrenal rest tumours (TART). These adrenal-like cells in the testes may grow in the presence of chronically elevated blood ACTH concentrations as occurs in poorly controlled CAH patients. However, several studies do not describe a clear correlation between hormonal control and tumour growth.

In this thesis we studied several aspects of TART in adult male CAH patients.
The most important questions were:
1. What are the functional features of testicular adrenal rest tumours in male CAH patients?
2. Which treatment options are effective?
For this purpose, we studied a group of 8 adult male CAH patients with longstanding bilateral TART and gonadal dysfunction. All patients were treated with testis-sparing surgery in an attempt to improve gonadal function. The removed tumour tissue was used for molecular investigations. We discuss the advances and side effects of medical treatment in an adult male CAH patient with bilateral TART, who was treated with short periods of dexamethasone in an attempt to reduce tumour size and improve gonadal function. Furthermore, we investigated the incidence of TART in relation to gonadal function in children with CAH and we describe a case of a girl with adrenal rests in her ovary. All investigations were performed in the Radboud University Nijmegen Medical Centre, The Netherlands.

General introduction

Chapter 1.1 gives a general overview of CAH. In **chapter 1.2** several aspects of fertility in male and female CAH patients are discussed.

Functional features and clinical consequences of testicular adrenal rest tumours in patients with congenital adrenal hyperplasia

Although there is evidence that TART have adrenal-like features, most studies describing functional aspects of the tumours only report on single patients. In our study we measured mRNA concentrations of the adrenal specific enzymes CYP11B1 and CYP11B2 and of ACTH and angiotensin II (AII) receptors in the 16 tumour samples of our patient group. In addition, in all but one patient, spermatic veins were canulated during surgery and blood samples were collected to measure the concentrations of the adrenal specific steroid 21-deoxycortisol (21DF) and also of 17-hydroxyprogesterone (17OHP) and androstenedione (A). The same parameters were measured in simultaneously taken peripheral blood. In **chapter 2.1** we describe the results of these studies. In 7 of the 8 patients biopsies of the surrounding testicular parenchyma were taken during enucleation of TART. In **chapter 2.2** we describe the results of the histological evaluation of TART and of the testicular biopsies in order to explain why testicular function did not improve after surgery.

Treatment options in male patients with congenital adrenal hyperplasia and testicular adrenal rest tumours

TART have no malignant features. However, because of the central localization of the tumours in the rete testis, they may compress the seminiferous tubules leading to obstructive azoospermia and irreversible damage of the testis. Because of these negative effects on gonadal function, medical and surgical strategies to treat the tumours have been developed.
Treatment with high doses of glucocorticoids may lead to suppression of ACTH secretion and reduction of tumour size. In **chapter 3.1** we describe a male CAH patient with bilateral TART and azoospermia who was treated with short periods of dexamethasone in an attempt to reduce tumour size and improve testicular function. Repeated spontaneous conception occurred after replacement of hydrocortisone therapy by short periods of dexamethasone (DXM) therapy causing decrease in tumour size and improvement of semen quality.
High doses of glucocorticoids however, may have several side effects and several studies showed that optimizing glucocorticoid therapy does not always reduce tumour size or restore

testicular function. Therefore, testis-sparing surgery has been proposed for the treatment of testicular adrenal rest tumours in CAH patients. We treated our patients with longstanding bilateral TART who did not respond to glucocorticoid therapy with testis-sparing surgery in an attempt to improve gonadal function. In **chapter 3.2** we describe the results of the clinical and biochemical evaluation of these patients before and after operation.

Value of magnetic resonance imaging in the evaluation of testis-sparing surgery in male patients with congenital adrenal hyperplasia and testicular adrenal rest tumours

The aim of testis-sparing surgery is complete removal of the tumours as well as preservation of normal testicular tissue. Therefore exact measurements of both tumor volume and testicular volume before and after surgery is of great importance. Because of the location in the rete testis detection of the tumours by palpation is only possible when tumour size is above 2 cm.
We performed pre- and postoperative testicular MRI in all operated patients to evaluate the usefulness of this imaging technique in the assessment of the volume of TART and residual testicular parenchyma before and after testis-sparing surgery. The results of the MRI measurements are described in **chapter 4.**

Incidence of testicular adrenal rest tumours in children with congenital adrenal hyperplasia

TART in post pubertal male CAH patients are frequently found, with a reported incidence of up to 94%. In contrast, the presence of TART in children is mostly described in case reports and only a limited number of studies describe the incidence of TART in children in a larger population. However, none of these studies focused on childhood age or provides information on gonadal function. To evaluate the incidence of TART in childhood we performed ultrasonography of the testes in all 34 male children (age 2 – 18 years) who regularly attend our outpatient clinic. Furthermore, we evaluated gonadal function by measuring blood FSH, LH, testosterone and inhibin B levels in CAH children in order to detect a possible negative influence of the presence of these tumours on gonadal function in childhood. The results of this study are described in **chapter 5**.

Ovarian adrenal rest tissue in congenital adrenal hyperplasia – a case report

Although the incidence of TART in male CAH patients is high, adrenal rests in female patients are very rare. It was suggested that ovarian rests are detected less frequently because

testicular tumours can be palpated or give pain and discomfort. However, in an earlier study by our group in thirteen female adult CAH patients no adrenal rest tumours were detected with MRI. In the literature two patients with ovarian rest tumours were described. In **chapter 6** we describe a third case of a young girl, who died in an Addisonian crisis due to undiagnosed CAH and in whom ovarian adrenal rest tissue was detected at post mortem examination.

Summary and general discussion

In **chapter 7** the main results of this thesis are summarized and several suggestions for future investigations concerning the etiology of TART and follow up and treatment of patients with TART are given.

Chapter 2

Functional features and clinical consequences of testicular adrenal rest tumours in patients with congenital adrenal hyperplasia

Chapter 2.1

Testicular adrenal rest tumours in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency show functional features of adrenocortical tissue

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Abstract

Context: In male patients with congenital adrenal hyperplasia (CAH) testicular adrenal rest tumours (TART) are frequently found which may interfere with gonadal function.

Objective: Determining steroid producing features of TART.

Design: Descriptive.

Setting: University Medical Centre.

Patients: Eight adult CAH patients with bilateral TART treated with testis-sparing surgery.

Interventions: In all but one patient spermatic veins were canulated during surgery and blood samples collected to measure the adrenal specific steroid 21-deoxycortisol (21DF) and 17-hydroxyprogesterone (17OHP) and androstenedione (A). Same parameters were measured in simultaneously taken peripheral blood. mRNA concentrations of adrenal specific enzymes CYP11B1 and CYP11B2 and ACTH and angiotensin II (AII) receptors were measured in tumours tissue.

Main outcome measures: Adrenal specific steroids/enzymes.

Results: 21DF, 17OHP and A levels were measurable in all spermatic vein samples. The ratio (mean + SD) between spermatic vein and simultaneously taken peripheral blood samples was 37.8 + 56.3 (21DF), 132.0 + 249 (17OHP) and 57.0 + 68.2 (A). CYP11B1, CYP11B2 and ACTH and AII receptor mRNAs were detected in all tumours with a strong correlation between ACTH receptor mRNA in tumours and 21DF ($r = 0.85$; $p = 0.015$), 17OHP ($r = 1$; $p = 0.01$) and A ($r = 0.89$; $p = 0.007$) concentrations in peripheral blood.

Conclusion: TART produce adrenal specific steroids and express adrenal specific enzymes and ACTH and AII receptors confirming the strong resemblance with adrenal tissue. As AII receptors are present in tumour tissue it can be hypothesized that AII may be an additional factor responsible for TART growth.

Introduction

In 1940 Wilkins et al. first reported the presence of a testicular tumour in a male patient with congenital adrenal hyperplasia (CAH) (1). Two recent studies found that the prevalence of testicular tumours in these patients is remarkably high (2,3). The tumours are almost always bilaterally present and have benign features, but because of their location in the mediastinum testis they can lead to obstruction of the seminiferous tubules leading to gonadal dysfunction and infertility. The testicular tumours are thought to arise from aberrant adrenal cells in the testes that are stimulated by ACTH. Therefore, they are called testicular adrenal rest tumours (TART). However, until now the aetiology and functional features of the tumours are not completely known. Microscopically, the tumours show features of steroid producing tissue but the histological differentiation between tumours derived from Leydig cells and from adrenocortical cells is difficult (4-6).

The clinical observations that high doses of glucocorticoids can reduce tumour size, most probably due to suppression of ACTH secretion, and that tumour growth may be promoted in conditions where ACTH concentration is high, such as in poorly controlled CAH patients or in patients with Nelson's syndrome or Addison's disease, suggests the presence of ACTH receptors on tumour cells (7-11). However, intensifying of glucocorticoid treatment with suppression of ACTH secretion is not always successful in reducing tumour size and even in well-controlled CAH patients, with normal or suppressed plasma ACTH levels, testicular adrenal rest tumours are found (2,3,12). Therefore, most probably other unknown factors contribute to tumour growth.

In the past a limited number of *in vivo* and *in vitro* studies, mainly in single patients, were performed to investigate the aetiology and the functional features of testicular adrenal rest tumours in CAH patients. Clark et al. described the presence of the adrenal specific enzyme CYP11B1 (11ß-hydroxylase) in tumour tissue of a single CAH patient with testicular adrenal rest tumours (13). Bercovici et al. demonstrated the presence of adrenal specific steroids in one testicular adrenal rest tumour (14). The presence of adrenal specific 11ß-hydroxylated steroids such as 21-deoxycorticosterone (21DB) and 21-deoxycortisol (21DF) in blood taken from the gonadal veins is reported in three single cases (14-16). This indicates the presence of adrenal-like tissue in the testes of these CAH patients with 21-hydroxylase deficiency, because these steroids can only be synthesized by adrenal specific 11-hydroxylation, without

the need of the deficient 21-hydroxylation step. In the present study we measured the concentrations of the adrenal specific steroid 21DF and of 17-hydroxyprogesterone (17OHP) and androstenedione (A) in blood taken from the spermatic veins during testis-sparing surgery in seven male CAH patients with bilateral TART. Furthermore, we measured the mRNA expression of the adrenal specific enzymes CYP11B1 and CYP11B2 as well as of ACTH and angiotensin II (AII) receptors in 16 testicular tumours of eight patients by quantitative PCR. We demonstrate that the testicular tumours in CAH patients show functional features of adrenocortical tissue, which is in line with the hypothesis that they are derived from aberrant adrenal cells.

Patients and methods

Patients

Eight male CAH patients (mean age 31 years; range 23 to 51) with bilateral TART were selected for testis-sparing surgery because of infertility (n=5), poor hormonal control despite rigorous treatment (n=2), pain or discomfort (n=2) or hypogonadism (n=2). Five patients (no.1, 2, 4-6) had been treated by intensifying the glucocorticoid therapy (which was mostly with dexamethasone) in the past to reduce tumour size and improve testicular function without success suggesting the development of fibrotic or autonomous tissue within the tumour. Three patients refused intensifying the glucocorticoid treatment. Written informed consent was obtained from all patients. The study was approved by the local Ethic Committee. The patients' characteristics are listed in table 1. The diagnosis of CAH due to 21-hydroxylase deficiency was confirmed by mutation analysis in all patients. Sample preparation and the method used for mutation analysis were performede as described earlier (17). All but one patient had the classic salt-wasting (SW) form of CAH and were treated with glucocorticoids and mineralocorticoids since the neonatal period. One patient had the simple virilising (SV) form of CAH diagnosed at the age of five years old.

In all patients' biochemical and semen analyses were performed before and after operation to evaluate pituitary-gonadal function. All patients were azoosperm or oligosperm with low blood inhibin B levels before operation. In three patients (no. 1,2 and 5) hypogonadotropic hypogonadism was present due to elevated serum androstenedione/estrone levels in these patients (table 2). The results of the biochemical analyses have been described in detail elsewhere (18). For comparison the same patient numbers are used in the current paper.

Spermatic vein sampling

Testicular tumour enucleation took place under general (n=1) or loco-regional (n=7) anesthesia. Just before operation all patients received 2.5 mg dexamethasone (DXM) intravenously as stress medication. In patients 1 – 4, the operation started on the left side, in patients 5 – 8 the operation started on the right side. Via an inguinal incision and after opening of the inguinal canal, the spermatic cord was exposed. During this procedure special care was taken not to manipulate the testis, in order to prevent unwanted secretion of hormones into the circulation. The spermatic vein was canulated and blood samples were collected to measure 21DF, 17OHP and A concentrations.

Simultaneously, peripheral blood was collected from a cubital vein to measure the same hormones. The same procedure was performed at the other side after finishing the operation on the first side. A second simultaneous peripheral blood sample was not taken. ACTH stimulation tests or DXM suppression tests were not performed during spermatic vein canulation to avoid prolonged anesthesia in the patients. Patient 1 was operated without spermatic vein sampling. In patient 7, the spermatic veins at the right side were atrophic and the volume of collected blood was not sufficient for biochemical analyses. All sera were stored at – 20 °C until measurements.

Tumour tissue preparation

All removed tumour tissue was investigated macroscopically and microscopically. A portion of the tumour was fixed in 10% buffered formalin. Tissue sections of 5 µm were cut and stained with haematoxylin & eosin and with Von Giesons elastin stain. Another portion of the tumour was frozen immediately in liquid nitrogen and stored at – 80 °C until processing.

Hormone assays

Methods of measuring serum LH, FSH, testosterone, inhibin B and estrone concentrations were described in detail in an earlier publication (18). 17OHP and A concentrations in serum were measured by radioimmunoassay (RIA) after extraction and paper chromatography (19,20). For 17OHP the within asssay CV was 6.1% and the between assay CV 8.5% at 4.9 nmol/l.

Table 1. *Age, HSDS-THSDS, BMI and medication at the time of testicular surgery, phenotype, mutation analysis and operation indication in eight male CAH patients with bilateral testicular adrenal rest tumours before testis-sparing surgery*

P	Age (Years)	Phenotype[a]	Allele 1[b]	Allele 2[b]	HSDS-THSDS[c]	BMI (kg/m^2)	Daily glucocorticoid therapy (mg/m^2)[d]	Daily mineralocorticoid therapy (µg)[e]	Operation indication[f]
1	24	SW	Large deletion or conversion	Large deletion or conversion	-2.8	27.4	32.2 (HC 20-20-20 mg)	400	1,2
2	29	SW	Large deletion or conversion	Large deletion or conversion	-0.7	25.7	16.0 (HC 20-10 mg)	125	1,2
3	23	SW	IVS2-13A/C>G	IVS2-13A/C>G	-0.7	25.6	8.2 (HC 8-4 mg, DXM 0.1 mg)	62.5	2
4	32	SW	IVS2-13A/C>G	IVS2-13A/C>G	n.a	28.3	16.9 (HC 25-10 mg)	100	2,3
5	26	SW	IVS2-13A/C>G	IVS2-13A/C>G	-1.0	38.2	10.8 (HC 10-5-10 mg)	62.5	1,4
6	51	SV	I172N	Large deletion or conversion	-2.95	29.0	16.2 (HC 20-10 mg)	-	5
7	31	SW	Large deletion or conversion	Large deletion or conversion	-1.3	27.0	30.1 (HC 25-40 mg)	125	2
8	26	SW	IVS2-13A/C>G	IVS2-13A/C>G	-2.9	23.7	12.1 (DXM 0.5 mg)	62.5	2,3

P = patient number

[a]SW = classic salt wasting CAH; SV = classic simple virilising CAH.

[b]Nucleotides are numbered according to Higashi's functional *CYP21* sequence (35). To detect a large deletion or conversion southern blotting was used (17).

[c]Height is expressed as SDS and corrected for target height SDS (HSDS-THSDS)

[d]Doses of dexamethasone were converted to hydrocortisone equivalents (1 mg dexamethasone = 40 mg hydrocortisone)

[e]Mineralocorticoid medication (9-α-fluorohydrocortisone acetate) was taken in one to three doses.

[f]1 = poor hormonal control, 2 = infertility, 3 pain/discomfort, 4 = hypogonadotropic hypogonadism, 5 = hypergonadotropic hypogonadism

Table 2. *Serum levels of FSH, LH, testosterone, inhibin B, estrone, 17-hydroxyprogesterone (17OHP), androstenedione (A), ACTH and renin as measured three days before testis-sparing surgery at 9 a.m., before taking the morning dose of glucocorticoid in 8 male CAH patients with bilateral testicular adrenal rest tumours*

P	*FSH* (U/l)[a]	*LH* (U/l)[b]	*Testosterone* (nmol/l)[c]	*Inhibin B* (ng/l)[d]	Estrone *(pmol/l)*[e]	*17OHP* (nmol/l)[f]	*A* (nmol/l)[g]	*ACTH* (pmol/l)[h]	*Renin* (mU/l)[i]
1	<0.2	<0.2	37.0	nd	2700	720	100	***156.0***	***1012.0***
2	0.6	<0.2	14.0	76	1100	480	86	***180.0***	***46.0***
3	8.6	5.2	17.0	65	140	26	1.5	***9.1***	***39.0***
4	15.9	2.9	13.0	47	650	367	14	***42.2***	***119.0***
5	<0.2	<0.2	9.8	80	1400	865	50	***270.0***	***94***
6	55.2	44.9	7.1	10	210	5.1	0.9	***33.0***	***103***
7	39.3	12.3	18.0	9	200	4.3	1.2	***0.5***	***< 0.3***
8	6.3	5.6	20.0	5	230	10	2.2	***5.8***	***24***

P = patient number, 17OHP = 17-hydroxyprogesterone, A= androstenedione.

Normal values of our laboratory:

[a]FSH 1.5 – 11 U/l

[b]LH 1.4 – 8.5 U/l

[c]Testosterone 11 – 45 nmol/l

[d]Inhibin B 150 – 400 ng/l

[e]Estrone 65 – 220 pmol/l

[f]17OHP 2.0 – 10.8 nmol/l

[g]A 1.4 – 9.7 nmol/l

[h]ACTH 2.2 – 13.2 pmol/l

[i]Renin 5 – 75 mU/l

For A the intra-assay coefficient of variation (CV) was 4.9% and the inter-assay CV 7.6% at 4.2 nmol/l. Serum 21DF was assessed by RIA after prepurification by means of HPLC of ether extracts of the samples, including correction for procedural losses.

To summarize briefly, ^{3}H-21DF was added before extraction to correct for procedural losses. A Hypersil Gold column with a mobile phase consisting of methanol/water (47/53) gave full separation between 21DF and the potentially crossreacting steroids cortisol, corticosterone and 11-desoxycortisol. The ^{3}H-21DF-containing fractions were evaporated to dryness and dissolved in ethylene glycol-water. The recovered radioactivity was measured by liquid scintillation counting of an aliquot from the eluate. Subsequently, ^{3}H-21DF tracer and antiserum (raised against cortisol 21-hemisuccinate-BSA) were added and after incubation, free and bound tracer were separated by means of dextran-coated charcoal. The antibody-bound radioactivity was assessed by liquid scintillation counting of the supernatant. The calculations were performed by software specially designed to correct for the contribution of mass and radioactivity of the recovery tracer in the RIA. Increasing cortisol levels up to 5 µmol/l revealed an overall contribution of cortisol in the measured 21DF of less than 0.1%. Additions to serum of 21DF up to 35 nmol/l were fully recovered (102±1.8%). The detection limit was 0.16 nmol/l, when using a sample volume of 0.5 ml. Between-run CV was 7.8% at a level of 10.6 nmol/l. In 32 healthy volunteers (16 male, 16 female) values up to 1.8 nmol/l were found. In 3 of these healthy individuals, 21DF levels were below the detection limit of the assay. ACTH was measured by a two-step immunoradiometric assay (IRMA, Dynotest BRAHMS, Berlin, Germany) based on two monoclonal antibodies directed against different antigenic determinants on the ACTH 1-39 molecule. Plasma renin was measured by immunoradiometric assay (IRMA) provided by CIS bio International (Gif-sur-Yvette, France). Within- and between-run CVs were 7.4 and 7.2% at 6.8 mU/l, 6.2 and 2.6% at 37.4 mU/l, 1.3 and 4.7% at 216 8 mU/l. Reference values are 7-75 mU/l.

Molecular analysis

RNA extraction. Tissues stored at –80 ºC were pulverized using a microdismembrator (Braun, Melsungen, Germany) and kept in liquid nitrogen until RNA isolation. Total RNA was isolated from 20 mg of tissue powder using the RNeasy mini kit (Qiagen, Hilden, Germany) with on-column DNase-I treatment. Quality of the RNA was checked by examining ribosomal RNA bands after agarose gel-electrophoresis, and by amplifying 3 housekeeping genes as a control (see below) (21). RNA concentrations were determined

from the spectrophotometric absorption at 260 nm using the Genequant (Amersham, Eindhoven, the Netherlands).

RT-PCR. Purified total RNA (1.0 µg) was denatured for 10 min at 70 °C, and immediately cooled on ice. Reverse transcription was performed using the Reverse Transcription System (Promega Benelux B.V., Leiden, the Netherlands) according to the manufacturer's protocol. After annealing of random hexamers for 10 min at 20 °C, cDNA synthesis was performed for 60 min at 42 °C followed by an enzyme inactivation step for 5 min at 95 °C. Quantitative PCRs for CYP11B1, CYP11B2, ACTH receptor, AII receptor and PBGD were performed using Sybr Green Master Mix (PE Applied Biosystems, Nieuwerkerk a/d lJssel, the Netherlands) in a volume of 25 µl. The primers used were: CYPIIB1-fw ggc aga ggc aga gat gct g, CYPIIB1-rev tct tgg gtt agt gtc tcc acc tg, CYPIIB2-fw ggc aga ggc aga gat gct g, CYPIIB2-rev ctt gag tta gtg tct cca cca gga, ACTHrec-fw cga tcc cac acc agg aag at, ACTHrec-rev tca gtg tga tgg ccc ctt tc, AIIrec-fw cct cgc tgt ggc tga ttt act c, AIIrec-rev ctt tgc aca tca cag gtc caa, PBGD-fw cat tgc tgg taa cgg caa tg, PBGD-rev gta cga ggc ttt caa tgt tg.

Assays for HPRT and beta-actin were performed using pre-developed assay reagents (PE Applied Biosystems) in Universal Taqman Mix (PE Applied Biosystems). Comparison of the potential normalizing genes PBGD, HPRT and beta-actin showed that beta-actin was the most consistent within and between tissues. Therefore, all values are shown as relative numbers of molecule gene of interest over molecule of beta-actin. As no absolute calibrator was used, all values are in arbitrary units. Amplifications, with denaturation at 95 ºC for 10 min, and 40 cycles of 15 sec at 95 ºC (melting) and 60 sec at 60 ºC (annealing and elongation), were performed on an ABI Prism 7700 Sequence detection system (PE Applied Biosystems).

Statistical analysis

Data were expressed as mean + SD. To compare different blood samples within patients paired t-test was used to determine statistical significance. Intergroup differences were tested using non-parametric tests and Spearman rank correlation for correlation between parameters. A p value < 0.05 was considered significant (2-sided). None of the parameters investigated showed a significant difference in the mRNA levels of tumours derived from the left and right testis of an individual patient. Therefore, for statistical analyses the mean value of the measured parameters of an individual patient was used.

Results

Histopathology

The mean weight of the tumours enucleated from the first testes was 10.1 ± 11.2 grams (mean ± SD; range 0.5 – 27.4) and from the second testes 8.1 ± 7.3 grams (range 1.3 – 18.9) (table 3). Macroscopically, all tumours were firm and multilobular with a yellow to tan colour on cut surface and narrow bands of fibrous tissue. Microscopically, the tumours were sharply demarcated but unencapsulated and consisted of sheets or confluent cords of large polygonal cells with abundant eosinophilic cytoplasm, separated by dense fibrous tissue strands. Reinke crystals were absent.

Spermatic vein sampling

The results of the spermatic vein samplings are listed in table 3. In all patients variable levels of 21 DF, 17OHP and A were measured. The mean 21DF concentration was 57.5 ± 49.4 nmol/l (mean ± SD; range 8.5 -150) in the first canulated spermatic vein and 34.1 ± 33.1 nmol/l (range 1.5 – 87) in the second canulated spermatic vein. Although the 21DF concentrations in the second spermatic vein were lower than in the first canulated vein, probably because of ongoing suppression of ACTH due to DXM given just before surgery, the difference between the two measurements was not significant ($p = 0.09$).

The mean 21DF concentration in peripheral blood was 5.4 ± 8.0 nmol/l (range 0.3 – 22) and was significantly lower than the concentration in simultaneously taken first spermatic vein blood ($p = 0.02$). The mean ratio (± SD) of 21DF concentration in blood of the first cannulated spermatic vein and the simultaneously collected peripheral blood was 37.8 ± 56.3 ($p = 0.02$). The mean 17OHP and A levels in the first canulated spermatic vein were also significantly higher compared with those in simultaneously taken peripheral blood ($p = 0.001$ and $p = 0.01$ respectively). There were no significant correlations between the concentrations of the steroids measured in the spermatic veins and tumour weight.

Table 3. *Tumour weight and concentrations of 21DF, 17OHP and A measured in spermatic veins and peripheral blood in seven of eight CAH patients with bilateral testicular adrenal rest tumours.*

P	**Tumor weight (gram)**		**21 DF (nmol/l)**				**17 OHP (nmol/l)**				**A (nmol/l)**			
	First	Second	P	I	II	*Ratio I/P*	P	I	II	*Ratio I/P*	P	I	II	*Ratio I/P*
1	nd	nd	nd	nd	nd	*nd*	nd	nd	nd	*nd*	nd	nd	nd	*nd*
2	13	11	5.1	52	59	*10.2*	16	140	130	*8.75*	4.4	21	9.1	*4.8*
3	4.3	5.5	1.4	65	3.6	*46.4*	3	170	77	*56.6*	0.3	20	6	*62.5*
4	27.4	18.9	8.0	150	87	*18.8*	6	260	160	*43.3*	0.8	20	5.1	*26.3*
5	0.9	1.6	22.0	74	27	*3.4*	46	190	78	*4.1*	7.2	19	5.2	*2.6*
6	22.9	16.2	0.3	53	54	*160.6*	0.5	320	450	*640*	0.4	65	59	*185.7*
7	0.5	1.3	0.3	nd	1.5	*nd*	2.2	nd	140	*nd*	0.5	nd	11	*nd*
8	1.5	1.9	0.3	8.5	6.7	*25.8*	4	170	280	*42.5*	0.6	36	80	*65.5*
mean	10.1	8.1	5.4[a]	57.5	34.1	37.8	11.1[b]	208.0	187.0	132.0	2.0[c]	30.1	25.06	57.0
(SD)	(11.2)	(7.3)	(8.0)	(49.4)	(33.1)	(56.3)	(16.2)	(67.9)	(134)	(249)	(2.7)	(18.2)	(31.0)	(68.2)

P = patient number. First = first enucleated tumour, Second = second enucleated tumour. P = peripheral blood sample, I = first spermatic vein sample, II = second spermatic vein sample. The peripheral blood sample was taken simultaneously with the first spermatic vein sampling (I). 17OHP = 17 hydroxyprogesterone, A = androstenedione, 21 DF = 21 deoxycortisol. Nd = not determined. In patient 1 only tumour size was measured and spermatic vein sampling was not performed. In patient 7 spermatic veins on the right side were atrophic and could not be canulated.

[a] $p = 0.02$, [b] $p = 0.001$, [c] $p = 0.01$ (p value gives the Spearman's correlation between I and P of each parameter)

Table 4. *Spearman rank correlation between mean of both tumours in individual patients of ACTH receptor, AII receptor, CYP11B1 and CYP11B2 mRNA levels measured in removed tumour tissue by Q-PCR and 21DF, 17OHP and A measured in spermatic veins and simultaneously taken peripheral blood samples in eight CAH patients with bilateral adrenal rest tumours treated with testis-sparing surgery.*

		Spermatic vein samples			**Peripheral blood samples**			**mRNA levels (Q – PCR)**		
		21DF	17OHP	A	21DF	17OHP	À	AII	CYP11B1	CYP11B2
mRNA levels	ACTH rec	0.43	-0.35	-0.67	0.85	1	0.89	0.38	0.17	0.17
		(0.34)	(0.50)	(0.15)	(0.015*)	(0.01**)	(0.007**)	(0.35)	(0.69)	(0.69)
	AII rec	-2.86	-0.09	0.41	0	0.14	0.43	-	-0.26	-0.38
		(0.54)	(0.87)	(0.43)	(1.0)	(0.76)	(0.34)		(0.53)	(0.35)
	CYP11B1	0.21	-0.41	-0.32	0.37	0.32	0.14		-	0.95
		(0.65)	(0.43)	(0.54)	(0.41)	(0.48)	(0.76)			(< 0.01**)
	CYP11B2	0.43	-0.23	-0.64	0.52	0.32	0.07	-	-	-
		(0.34)	(0.66)	(0.17)	(0.23)	(0.48)	(0.88)			

Q-PCR = real-time polymerase chain reaction, ACTH rec = mean ACTH receptor mRNA level measured in tumour tissue, AII rec = mean AII receptor mRNA level measured in tumour tissue. CYP11B1 and CYP11B2 = mean enzyme mRNA level of CYP11B1 and CYP11B2 measured in tumour tissue.

21DF = 21-deoxycortisol, 17OHP = 17-hydroxyprogesterone, A = androstenedione.

Numbers express correlation coefficients, 2-sided p value in parentheses.

* Correlation is significant at the 0.05 level (2-sided), ** Correlation is significant at the 0.01 level (2 –sided)

Expression analysis

CYP11B1 and CYP11B2 mRNAs were clearly detectable in all tumour samples. The mean CYP11B1 level was 4.1 ± 3.1 (mean ± SD; range 0.7 – 8.5) and the mean CYP11B2 level was 3.4 ± 2.6 (range 0.4 – 7.5) with a strong correlation between these two measurements ($r = 0.95$; $p < 0.01$). mRNA of ACTH receptors (0.2 ± 0.2;mean ± SD; range 0 – 0.6) and AII receptors (0.19 ± 0.032;range 0.00032 – 0.112) were also present in all tumour samples.
The correlations between mRNA expression of ACTH receptors, AII receptors, CYP11B1 and CYP11B2 and the steroid hormones 21DF, 17OHP and A measured in the spermatic veins and peripheral blood are listed in table 4. There was a strong positive correlation between tumour ACTH receptor levels and 21DF ($r = 0.85$;$p = 0.015$), 17OHP ($r = 1$;$p = 0.01$) and A concentration ($r = 0.89$; $p = 0.007$) measured in peripheral blood suggesting a strong influence of hormonal control on ACTH receptor levels in the tumours. No other significant correlations were found.

Discussion

Testicular adrenal rest tumours in male CAH patients are of great interest because of their high prevalence and severe consequences for gonadal function. Although several studies describe finctional characteristics of these tumours, mainly in case reports, our study is the first providing functional features of a series of 16 testicular tumours from eight adult CAH patients.
Histologically, all tumours showed a similar appearance with microscopically specific features of steroid producing cells in agreement with previous studies (4-6). In all but one patient, in whom canulation of the spermatic vein was not completely successful; the concentration of the adrenal specific steroid 21DF in the spermatic veins was significantly higher than the concentration in peripheral blood samples, which suggests local production of these steroids in the testes. Additionally, our study clearly shows the presence at the mRNA level of the adrenal specific enzymes CYP11B1 and CYP11B2 and of ACTH and AII receptors in all testicular tumours, strongly suggesting that the tumours consists of adrenal-like tissue.
Ectopic adrenocortical tissue is found in up to 50% of neonates and usually atrophies during childhood (22). Most ectopic adrenal tissue is found in the vicinity of the adrenals around the celiac axis and in the testes (surgery or autopsy findings). In the embryological period

steroidogenic cells destined to become adrenal and gonadal cells derive from neighbouring areas of the coelomic epithelium and are morphologically identical. Separation of the cells takes place at approximately week eight of gestation and further development of the cells depends on the expression of specific transcription factors (23,24). During further development "adrenal" cells can migrate together with the descending testis. Adrenal rests within the testis occur in 7.5 to 15% of neonates and normally regress in early infancy (25,26). However, in CAH patients it is believed that these cells can persist and proliferate with preservation of adrenal like hormone producing properties.

Functional adrenal zonation of testicular adrenal rests due to zona specific expression of enzymes involved in steroid biosynthesis has never been described. It is known that in the human adrenal gland CYP11B1 is expressed in high levels in the zona fasciculata/reticularis where it catalyzes the 11ß-hydroxylation of 11-deoxycortisol to cortisol (27). CYP11B2 is exclusively expressed in the zona glomerulosa where it is responsible for the final step of the aldosterone synthesis pathway (28,29). The presence of CYP11B1 in the zona glomerulosa is controversial (30). So, the presence of CYP11B1 and CYP11B2 in tumour tissue of all patients in our study group suggests that the tumours may have functional features of both adrenal zona fasciculata and glomerulosa cells. Furthermore, our study shows that these tumours are very heterogeneous with respect to steroid hormone production and that at least at the mRNA level they contain varying amounts of steroid producing enzymes and ACTH and AII receptors.

The factors that are responsible for growth of adrenal rest tissue in CAH patients are not fully understood. TART are often found in patients with poor hormonal control and high ACTH levels, suggesting that ACTH is a dominant factor in tumour growth (2,3). In the complete absence of 21-hydroxylase activity, plasma levels of ACTH are extremely high from early prenatal life, probably explaining the higher incidence of testicular tumours in SW CAH patients compared with SV CAH patients (12). However, in several studies no correlation was found between ACTH levels and tumour growth (2,3,12). Therefore, most probably other factors contribute to tumour growth.

In our study we found mRNA expression of AII receptors in all testicular tumours. These findings are in agreement with the study of Clark et al. who described AII receptor concentrations in a testicular adrenal rest tumour of a CAH patient similar to that in normal adrenal tissue (13). It is known that AII has a strong trophic effect on the adrenal gland, especially on the zona glomerulosa (31-34). These trophic effects were studied in detail by Chatelain et al. in adult rats showing that water deprivation resulted in high AII levels without

affecting ACTH levels and in increase of adrenal zona glomerulosa weight (31). Inhibition of AII production by angiotensin converting enzyme inhibitors significantly decreased adrenal weight suggesting that AII is an important factor in stimulation of adrenal growth. AII markedly increases levels of both CYP11B1 and CYP11B2 mRNA, whereas ACTH causes an acute increase of CYP11B1 mRNA levels without an effect on CYP11B2 transcription (31).

We hypothesize that tumour growth in CAH patients may not only be stimulated by elevated ACTH concentrations but also by elevated AII levels, which are present in SW patients with poor hormonal control. Interestingly, in late onset CAH patients, without clearly elevated ACTH or AII levels, testicular tumours are never described. Further studies are necessary to study the effect of AII on growth of testicular tumours in CAH patients.

In summary, TART in CAH patients produce adrenal specific steroids and contain adrenal specific enzymes confirming the adrenal-like properties of the tumours. Based on the presence of AII receptors we hypothesize that AII may be an additional factor responsible for tumour growth in patients with poor hormonal control. Further investigations will be necessary to determine the role of AII in testicular tumour growth.

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Chapter 2.2

Testicular adrenal rest tumours in patients with congenital adrenal hyperplasia can cause severe testicular damage

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Abstract

Study objective

To evaluate the histological features of testicular tumours and residual testicular parenchyma in male patients with congenital adrenal hyperplasia (CAH) and longstanding bilateral testicular adrenal rest tumours (TART).

Design

Descriptive study.

Setting

Radboud University Nijmegen Medical Centre, The Netherlands.

Patients

Seven male CAH patients with longstanding bilateral TART treated with testis-sparing surgery.

Interventions

Enucleation of TART and taking biopsies of the surrounding testicular parenchyma.

Main outcome measures

Description of the histological features of TART and residual testicular parenchyma.

Results

All tumours had a similar histological appearance with sheets of polygonal cells separated by dense fibrous tissue with focal lymphocytic infiltrates and without Reinke crystals. All biopsies showed a decrease in tubular diameter with peritubular fibrosis and in four patients tubular hyalinization. The germinative layer showed decreased spermatogenesis and reduced Johnsen scores.

Conclusions

TART can lead to end-stage damage of testicular parenchyma most probably due to longstanding obstruction of the seminiferous tubules. Therefore, treatment at an early stage is advised.

Introduction

Testicular adrenal rest tumours (TART) are a common complication in adult male patients with congenital adrenal hyperplasia (CAH) (1,2). The tumours are always benign with a typical bilateral location near the mediastinum testis (3-8). Because of their location, the tumours can lead to mechanical obstruction of the seminiferous tubules (9-11). Different medical and surgical treatment strategies are available to treat the tumours and to prevent gonadal dysfunction. However, the beneficial effects of medical treatment are described mostly in case reports with different outcomes depending on patient selection, type of CAH and treatment choice (12-17). Furthermore, in a recent study we showed that testis-sparing surgery had no beneficial effect on gonadal function in patients with longstanding bilateral TART (18). This may be explained by the irreversible damage of the residual testicular tissue, for example due to chronic obstruction of the seminiferous tubules.

So far, no detailed studies have been reported about the quality of the residual testicular parenchyma in patients with TART. In most case reports histological descriptions focussed on testicular tumours and only a few reports gave some information about the residual testicular tissue as well. Battaglia et al. reported smaller seminiferous tubules with thickening of the basal membrane in one CAH patient with TART (16). Bonaccorsi et al. described partial or total hyalinization of the seminiferous tubules in one CAH patient with TART (12). Knudsen et al. described atrophic testicular tissue with hyaline thickening of the tubular basal membrane in the testicular biopsy of a 26-year-old infertile male patient with TART who had not been diagnosed as a CAH patient before (19).

We treated seven male CAH patients with longstanding TART with testis- sparing surgery (18). Here we describe the results of the histological evaluation of TART in these patients. Furthermore, we investigated the histology of the residual testicular tissue in order to explain why testicular function did not improve after surgery in these patients.

Patients and methods

Patients

Seven male patients with CAH due to 21-hydroxylase deficiency and longstanding, bilateral TART were treated with testis-sparing surgery because of infertility (n = 5), poor medical

control (n = 2) and testicular pain and discomfort (n = 2). The age of the patients was 30 ± 8.9 years (mean ± SD; range 23-51 years). Four of these patients (no. 3, 5, 6 and 7) had been treated with high doses of glucocorticoids to reduce tumour size without success. All but one patient had the classic salt wasting form of CAH; one patient (no.7) had the simple virilising form of CAH. The diagnosis CAH was confirmed by mutation analysis in all patients. Semen analysis was carried out in five of the seven patients before and after surgery according to the World Health Organization (WHO) guidelines (20). Azoospermia was found in four patients (no.1,2,3,7) and oligospermia in one patient (no.6). This condition did not improve even after surgery. All patients had low inhibin B concentrations before and after surgery. A detailed description of the biochemical investigations before and after surgery has been given earlier (18). The tumours were successfully enucleated in all patients without evidence of residual tumour on MRI.

Histopathology

All removed tumour tissue was investigated macroscopically and microscopically. Biopsies of testicular parenchyma surrounding the tumours were taken during the operation in all patients. Testicular biopsies were fixed in 10% buffered formalin. Tissue sections of 5 μm were cut and stained with haematoxylin & eosin and with Von Giesons elastin stain. Testicular biopsies were scored according to Johnsen with a score varying from 0 to 10 (21).

Results

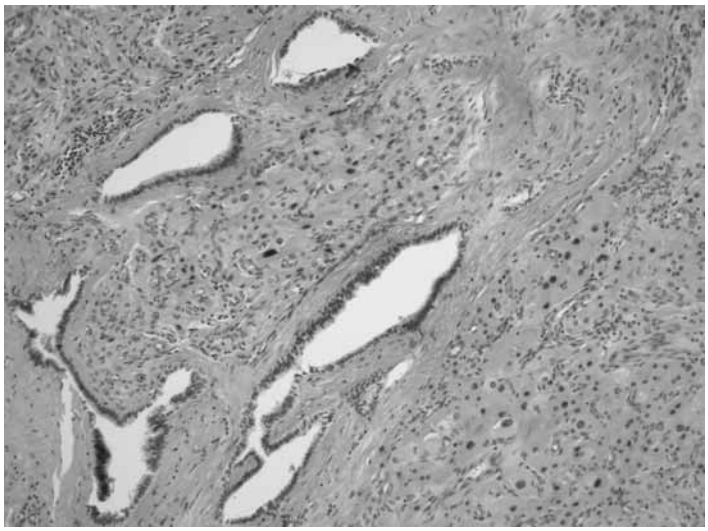
A. Testicular tumours

Tumour weight and histological characterization are listed in table 1. Weight of the tumours in the left testes was 9.8 ± 9.9 grams (mean ± SD; range 1.3 – 27.8) and in the right testes 8.7 ± 9.1 grams (range 0.45 – 22.9). Macroscopically, all tumours were firm and multilobular with a yellow to tan color on cut surface and narrow bands of fibrous tissue.

The maximal diameter of the tumours varied from 1 to 7 cm. On microscopy, the tumours were sharply demarcated but unencapsulated and consisted of sheets or confluent cords of large polygonal cells with abundant eosinophilic cytoplasm, separated by dense fibrous tissue strands.

In all patients the tumours were located in the hilar area of the testis with compression of the rete testis (fig.1).

Figure 1. *Testicular adrenal rest tumour growing into rete testis (RT) (HE, original magnification x 200).*



Within the tumour fields, there were regular thin fibrovascular septa, but a zonal arrangement was absent. Focal lymphocytic infiltrates were present. The cytoplasm of the tumour cells contained different amounts of lipofuscin pigment. Cells with large, clear intracytoplasmic vacuoles were focally found. Reinke cristals were absent. The nuclei were round with a central prominent nucleolus and showed clear variation in size with frequent intranuclear cytoplasmic inclusions. Mitotic figures were rare.

In all patients the tumours were cellular, except in patient 5 in whom broad fibrous bands with abundant adipose tissue had replaced the tumour cells leaving only small aggregates of tumour cells filled with lipofuscin pigment.

B. Testicular biopsies

All biopsies from residual testicular parenchyma showed decreased spermatogenesis with reduced Johnsen scores (table 1). The germinative layer showed a decreased number of all types of germ cells with a clear reduction in the number of spermatides in six patients and complete absence of spermatides in one patient (no.1) where maturation was arrested at spermatocyte level).

Sertoli cells showed some vacuolization, but were not dysplastic. Leydig cells were present in

normal or slightly reduced numbers in 5 patients, pronounced in one patient (no.6) and not discernable in another patient (no.1).

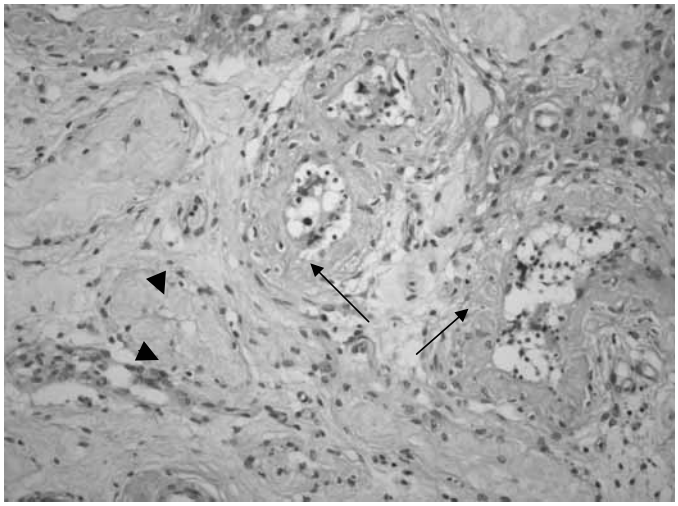
The tubular diameter was reduced in all cases, with a diameter of 139 ± 16 μm (mean ± SD; normal value: 193 ± 8 μm). In all patients there was a different degree of thickening of the tubular lamina propria with peritubular fibrosis and retained or increased elastic fibres. There was an increase in peritubular fibroblasts in four patients (no. 1,3,4,5). In four of the seven patients complete tubular hyalinization was focally present (no.1,2,3,5), being most severe in patient 5 who had been treated with high doses of glucocorticoids for a long time (fig.2).
There were no signs of ischemia, hemorrhage or vessel impairment. The presence of Leydig cells, increased peritubular cellularity and mosaic pattern of tubular hyalinization all favored end-stage testicular damage, most probably due to an obstructive origin.

Table 1. *Tumour weight and Johnsen score (21), mean tubular diameter and highest level of sperm maturation in testicular biopsies of seven male CAH patients with bilateral testicular adrenal rest tumours. A Johnson score of > 8 is associated with fertility.*

No.		Tumour weight (grams)	Testicular biopsy		
			Johnsen score	Mean tubular diameter (μm)	Highest level of sperm maturation
1	L	13.0	3.5	138	spermatocyte
	R	11.0	4.1	138	spermatocyte
2	L	4.3	2.3	140	Sertoli cell only
	R	5.5	6.4	153	Late spermatid
3	L	27.8	3.4	122	Late spermatid
	R	18.9	4.7	183	Primary spermatid
4	L	1.6	7.6	127	Late spermatid
	R	0.9	6.4	142	Late spermatid
5	L	16.2	2.4	nd	Late spermatid
	R	22.9	1.0	nd	No cells left
6	L	1.3	2.8	138	Late spermatid
	R	0.5	2.0	133	Sertoli cell only
7	L	1.9	7.2	133	Late spermatid
	R	1.5	3.3	125	Late spermatid

No = patient number, L = left testis, R = right testis, nd = not determined.

Figure 2: *Testicular biopsy of patient 5 showing seminiferous tubules with hypospermatogenesis and prominent peritubular fibrosis with increased number of peritubular fibroblasts (arrows), as well as tubular hyalinisation (arrow-head; original magnification x 200).*



Discussion

The occurrence of TART is an important complication in male CAH patients. The tumours have no malignant features and therefore, there seems to be no need to remove them at an early stage. However, because of the central localization of the tumours near the mediastinum testis, compression of the seminiferous tubules might lead to obstructive azoospermia and irreversible damage of the testes. We found a decreased tubular diameter and a varying degree of peritubular fibrosis and tubular hyalinization in the testicular biopsies of all our patients.

Tubular hyalinization is a general finding in end-stage testicular damage and is caused by massive deposition of collagen fibers inside the seminiferous tubules (22,23). In our patients, tubular hyalinization could have been caused by chronical obstruction of the seminiferous tubules. In the literature obstructive azoospermia is described mainly as a result of extra-testicular obstruction due to infections or surgical interventions mostly located at the epididymis or vas deferens (24-26). In these cases, adverse effects of the obstruction on the germinal epithelium or Leydig cells were not reported (25,27). This can be explained by the ability of the epididymis to become enlarged, to accommodate the sperm cells and to phagocytize and resorb spermatozoa (25).

In contrast, we found a severe decrease in the number of germ cells in all patients. It can be speculated, that in the case of large TART located in the mediastinum testis proximal to the

epididymis, the efferent flow in the seminiferous tubules is chronically obstructed without having the ability of compensatory dilatation of the epididymis. Longstanding obstruction of the seminiferous tubules could then lead to hypospermatogenesis and peritubular fibrosis. The irreversible end-stage is tubular hyalinization with obstruction of the lumen and complete loss of germ cells and Sertoli cells. In contrast to ischemic hyalinization, where a reduced number of Leydig cells are expected, the interstitium of our patients contained a normal or only slightly reduced number of Leydig cells. Therefore, TART may form a very specific cause of obstructive azoospermia, commonly not mentioned in the literature and with more severe clinical consequences.

Several treatment strategies to reduce tumour size with consequently reduction of tubular obstruction and improvement of gonadal function in patients with CAH and TART have been described (12-18). It is clear that in the case of irreversible damage of the testicular parenchyma due to the tumour, any attempt to reduce or remove the tumours is not successful in restoring fertility. Our study shows marked interstitial and peritubular fibrosis with severe reduced Johnsen scores in all biopsies suggesting end-stage testicular damage due to obstruction of TART.
In extra-testicular obstructive azoospermia, treatment of infertility with intracytoplasmatic sperm injection (ICSI) can be successful. This treatment option was also described in a male CAH patient with bilateral TART (10). Testicular biopsy taken at some distance from the site of the tumour showed normal testicular parenchyma. A possible explanation is that progressive damage of the testicular parenchyma due to tumour obstruction starts in the neighborhood of the tumours with a progressive involvement of the parenchyma further away of the tumours position. Therefore, not only the localization of the tumours but also the size of the tumours and the duration of the obstruction are important factors for the development of gonadal dysfunction.

Unfortunately, small tumours are difficult to detect by palpation because of their location in the mediastinum testis. In earlier studies we showed that ultrasonography appeared to be a good method for detection and follow-up of the tumours, especially when they are non-palpable (28). Therefore, regular ultrasonography starting during or even before puberty was advised to detect and treat the tumours at an early stage and prevent permanent gonadal damage. In the case of longstanding tumours in infertile CAH patients, a testicular biopsy may be helpful to evaluate the quality of residual testicular parenchyma. However, one should

realize that a testicular biopsy only gives information about a circumscriptive area of the testes. In our study we took all biopsies near the TART. Therefore, we did not have additional information about the whole residual testicular parenchyma. However, hormonal and semen analysis before and after surgery favored the diagnosis of irreversible testicular damage due to longstanding TART.

In summary, TART can lead to irreversible damage of testicular parenchyma, most probably due to obstruction of the seminiferous tubules. Therefore, despite the benign features, early treatment to remove the tumours or to reduce their size is advised.

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Chapter 3

Treatment options in male patients with congenital adrenal hyperplasia and testicular adrenal rest tumours

Chapter 3.1

Repeated successful induction of fertility after replacing hydrocortisone by dexamethasone in a patient with congenital adrenal hyperplasia and testicular adrenal rest tumours

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Abstract

Objective

To report repeated successful induction of fertility in an adult male patient with congenital adrenal hyperplasia (CAH) and testicular adrenal rest tumours (TART)

Design

Case report

Setting

Radboud University Nijmegen Medical Centre

Patient

A 23-year-old male CAH patient with bilateral TART and azoospermia.

Interventions

Changing glucocorticoid medication from 30 mg hydrocortisone to 0.75 mg DXM daily

Main Outcome Measures

Improved semen analysis

Results

TART were detected by ultrasound screening at the age of 23 years. Semen analysis showed azoospermia. In an attempt to decrease tumour size and improve testicular function, glucocorticoid medication was changed from hydrocortisone to equivalent dosages of dexamethasone. Azoospermia was quickly reversed and conception was achieved within 7 months after stopping oral contraceptives. Due to progressive weight gain and striae, medication was changed to hydrocortisone. He developed again azoospermia. Two years later the patient started again with DXM in the same dosage. Sperm concentration increased with progressive weight gain. Seven months later his wife became pregnant for the second time and DXM treatment was changed to hydrocortisone.

Conclusions

In male CAH patients due to testicular adrenal rest tumours Infertility may be reversible due to replacing hydrocortisone by short courses of equivalent dosages dexamethasone.

Introduction

In male patients with congenital adrenal hyperplasia (CAH), infertility is an important complication (1-4). In most cases infertility is related to the presence of testicular tumours, which can be frequently found: in two recent studies an incidence up to 90% is described (3-4). These tumours are thought to arise from aberrant adrenal cells in the testes that are stimulated by chronically elevated ACTH concentrations (5). Therefore they are called testicular adrenal rest tumours. The tumours are located in the mediastinum testes leading to obstruction and congestion of the seminiferous tubules. A second cause of gonadal dysfunction in male CAH patients may be suppression of the hypothalamic-pituitary-gonadal axis due to high blood concentrations of adrenal androgens (6-7). In the non-classic form of CAH infertility can be the sole symptom and consequently the diagnosis CAH may be established only at evaluation of infertility (8-11). Analysis of these patients reveals low levels of gonadotropines with (sub-) normal levels of testosterone and testicular volumes in the lower range suggesting inhibition of the pituitary-gonadal axis and failure of normal testicular maturation due to high levels of adrenal androgens. In these patients intensifying glucocorticoid therapy leads to normalization of gonadotropin levels by suppression of adrenal androgens with improvement of testicular function (8-11). Testicular tumours are not detected in these mild forms of CAH.

In CAH patients with testicular adrenal rest tumours, intensifying glucocorticoid therapy may lead to reduction of the tumour size by suppression of ACTH secretion thereby improving testicular function. Although case reports with successful pregnancy have been published some studies report failure of intensified glucocorticoid treatment and serious side effects after long-standing dexamethasone treatment (12-15).

We describe a case of repeated successful fertility in a male CAH patient with bilaterally testicular tumours who presented with azoospermia. Repeated spontaneous conception occurred after replacement of hydrocortisone therapy by short periods of dexamethasone (DXM) therapy causing decrease in tumour size and improvement of semen quality.

Case

The patient, born in 1975, presented at the age of 6 years with symptoms of increased growth velocity, pubic hair and penile enlargement. The diagnosis simple virilising (SV) CAH was

made based on hormonal analysis and was later confirmed by mutation analysis with a deletion/conversion in allele 1 and an I12N mutation in allele 2. He was treated with hydrocortisone and fludrocortisone with adequate suppression of the adrenal androgens during puberty. Bilateral testicular volume at the end of puberty was 18 ml estimated by Prader's orchidometer. His final height was 181.3 cm (-0.3 SDS). At the age of 23 years bilaterally testicular adrenal rest tumours were detected by ultrasound screening with a tumour size of 3.7x1.4x2.3 cm at the left side and 3.2x1.9x2.3 cm at the right side without palpable masses or complaints. At that time androstenedione levels were slightly elevated with normal levels of gonadotropines. Semen analysis showed azoospermia (table 1. Hydrocortisone dosage was 30 mg daily divided over 2 dosages (16 mg/m^2). In an attempt to decrease tumour size and to improve hormonal control and semen quality, medication was changed to equivalent dosages of DXM of 0.75 mg daily divided over 3 dosages. To suppress nocturnal ACTH secretion the last dose was given at 10.00 pm. Within three months semen analysis revealed improved sperm count. On ultrasound, tumours were reduced in size but still present in both testes. Androstenedione levels decreased to a lower range. However there was progressive weight gain with development of striae. Conception was achieved within 7 months after stopping oral contraceptives. Because of ongoing weight gain, glucocorticoid medication was changed to hydrocortisone. Androstenedione levels increased and he developed again azoospermia. Ultrasound of the testes was not repeated. Two years later the couple wanted a second pregnancy and the patient started with DXM in the same dosage as during earlier DXM treatment. Sperm concentration increased with again progressive weight gain. Seven months later his wife became pregnant for the second time. After pregnancy was achieved, DXM treatment was again changed to hydrocortisone.

Table 1. *Medication, body weight, hormonal and semen analysis and ultrasonographic evaluation of the testes in a male patient with SV CAH and bilaterally testicular adrenal rest tumours*

Date	**July 1998**	**July 1999**	**May 2000**	**September 2001**	**July 2002**	**August 2003**	**February 2004**	**June 2004**	
Medication	HC[a]20-10 mg F[b] 62.5 ug	DXM 0.75mg F 62.5 ug	DXM 0.75mg F 62.5 ug	DXM 0.75 mg F 62.5 ug	HC 20-10 mg F 62.5 ug	HC 20-20 mg F 62.5 ug	DXM 0.75 mg F 62.5 ug	HC 20–10 mg F 62.5 ug	
Weight (kg)	79.8	80.5	81.5	87	80.2	81.4	85.7	79	*Normal values*
Sperm Concentration[c] (x 10^6/ml)	< 0.1	10	50		1	<0.1	7		*≥ 20*
LH (U/l)	4	-	2.4	2.5	2.9	3.7	4.4	6.6	*1.4 – 8.5*
FSH (U/l)	5.7	-	11.9	11.1	10.1	10.7	19.2	9.2	*1.5 – 11.0*
Testosterone (nmol/l)	6.6	9.6	10	7	13	14	10	11	*11.0 – 45.0*
17OHP[6] (nmol/l)	7.9	2.5	2.2	1.5	19	51	2.2		*2.1 – 7.6*
Androstenedione (nmol/l)	9.6	0.57	0.68	0.33	1.3	6.2	0.59		*2.3 – 8.4*
ACTH (pmol/l)	2.1	< 1.0	<1.0	-	5.2	4.69	<1	-	*2.2 – 13.2*
Saliva 17OHP (nmol/l)				-	-		-		
- morning	5.8	0.06	0.049			4.7		14	*0.05 – 0.36*
- noon	1.6	0.05	0.026			0.39		6.5	
- evening	4.3	0.03	<0.016			0.19		0.77	
Saliva androstenedione (nmol/l)									
- morning				-	-		-		
- noon	2.3	0.07	0.12			0.55		1.2	*0.14 – 0.63*
- evening	1.2	0.09	0.061			0.23		0.47	
	1.9	0.08	0.051			0.11		0.22	
Ultrasound tumour size (cm)									
- Left testis	3.7x1.4x2.3	-	-	1.4x1.1x0.7	-	-	1.6x2.0	-	
- Right testis	3.2x1.9x2.3			1.4x1.3x2.0			2.4x1.6		
Comments	April 1999: stop HC start DXM 0.75 mg[d]		February 2001: stop OC[e]	September 2001: Pregnancy stop DXM start HC 20-10 mg		August 2003: stop HC start DXM 0.75mg	February 2004: Pregnancy stop DXM start HC 20-10 mg	October 2005: Weight 70 kg	

[a]HC = hydrocortisone. [b]F = 9 alfa – fluorohydrocortisone acetate (taken in one dose in the morning). [c]Semen analysis was performed according to the 1999 World Health Organisation laboratory manual (24). [d]DXM = Dexamethasone. DXM was dosed three times a day. Converting factor: 30 mg hydrocortisone = 0.75 mg DXM

[e]OC = oral contraceptives, [6]17OHP = 17-hydroxyprogesterone

Discussion

We describe a male patient with SV CAH and bilaterally testicular adrenal rest tumours. At the time of detection of the tumours, the patient was azoospermic. The normal FSH level in combination with a normal testicular volume and azoospermia was highly suspicious for tubular obstruction due to the presence of the testicular adrenal rest tumours.
Poor hormonal control with inadequate suppression of ACTH may be an important factor in the etiology of testicular adrenal rest tumours. By increasing the glucocorticoid dose, ACTH secretion will be suppressed and the adrenal rest tissue will be less stimulated, which may lead to shrinkage of the testicular tumours. Indeed a few case reports describe shrinkage of the testicular adrenal rest tumours accompanied by reversibility of infertility (16-17). However, high doses of glucocorticoids may also have severe side effects such as weight gain or the development of striae (15). Moreover this treatment does not always restore testicular function despite shrinkage of the tumour. (7). This can be explained by the development of irreversible fibrotic thickening of the peritubular layers in response to chronic mechanical obstruction of the seminiferous tubules (18). In our patient changing of the glucocorticoid medication led to improvement of sperm count indicating absence of serious tubular damage.

As mentioned earlier, elevated levels of androstenedione can lead to suppression of the hypothalamic-pituitary-gonadal axis contributing to impaired fertility. Testicular function is regulated by the pulsatile secretion of gonadotropin-releasing hormone (GnRH) and gonadotropines. It is known that high concentrations of testosterone act mainly at the hypothalamic level by decreasing the frequency of GnRH pulses, whereas elevated estrogens depress the amplitude of the LH and FSH peaks (18). Although the gonadotropin concentrations in our patient were within the normal range, the increase in FSH levels after intensifying glucocorticoid therapy suggests mild Sertoli cell dysfunction, which may become visible after suppression of androstenedione levels. Furthermore, it can be speculated that in our patient pulsatility of FSH secretion was impaired due to slightly elevated levels of androstenedione and oestron, contributing to subnormal Sertoli cell function.

In our case DXM was used to optimize medical treatment in the same dose equivalent as hydrocortisone (glucocorticoid conversion factor: 30 mg hydrocortisone = 0.75mg DXM) (19). As DXM has a longer half-life than hydrocortisone (36-72 h versus 8-12h), DXM may suppress the hypothalamic-pituitary-adrenal axis more effectively (20). Some studies describe

a strong suppressive effect of DXM even in a lower dose (0.25 – 0.5 mg daily). Unfortunately, DXM may also give rise to serious side effects such as weight gain and striae. Our case report demonstrates that changing glucocorticoid therapy to DXM can improve testicular function but should be used as short as possible in order to avoid serious side effects. If tumour size is decreased without improvement of fertility, irreversible damage of the seminiferous tubules due to hyalinization and fibrosis might have occurred (18). A testicular biopsy may help to prove this diagnosis. If tumour size does not decrease, surgical intervention may be considered. Two studies describe testis-sparing enucleation of the tumour (21-22). However whether improvement of gonadal function after surgery occurs remains unclear. Therefore surgery has to be reserved to patients who do not respond to conservative treatment. If conservative treatment is not successful ICSI can also be considered to establish pregnancy (23).

In summary, our case demonstrates that infertility in CAH patients due to testicular adrenal rest tumours may be reversible due to replacing hydrocortisone by dexamethasone. Repeated periods of DXM treatment can already be successful, which may be preferred above chronic therapy in order to prevent serious side effects.

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Chapter 3.2

Testicular adrenal rest tumours in adult males with congenital adrenal hyperplasia: evaluation of pituitary-gonadal function before and after successful testis-sparing surgery in 8 patients

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Abstract

Context

In male patients with congenital adrenal hyperplasia (CAH) testicular adrenal rest tumours (TART) are frequently present. These tumours can interfere with testicular function. Intensifying glucocorticoid therapy does not always lead to tumour regression and improvement of testicular function. Recently testis-sparing surgery was introduced for treatment of TART.

Objective

Evaluation of tumour volume, symptoms and pituitary-gonadal function in male patients with CAH due to 21-hydroxylase deficiency and bilateral TART before and after testis-sparing surgery.

Setting

Radboud University Nijmegen Medical Centre, The Netherlands

Patients

8 adult male CAH patients with bilateral TART and infertility.

Interventions

Evaluation of testicular MRI, symptoms, fasting serum concentrations of ACTH, LH, FSH, inhibin B, 17-OH progesterone, androstenedione, testosterone and oestron, and semen analysis (6 of 8 patients) before, and 6 and 22 months after testis-sparing surgery.

Main outcome measures

Absence of residual tumour. Improvement of symptoms and pituitary-gonadal function**.**

Results

In none of the patients residual tumour was found on MRI after surgery. Two patients reported testicular pain and discomfort that disappeared after surgery. Parameters of pituitary-gonadal function did not improve after surgery: Semen analysis showed azoospermia (5 patients) or oligospermia (1 patient) without improvement and all patients had persistently low inhibin B concentrations.

Conclusion

Testis-sparing surgery did not improve pituitary-gonadal function despite successful removal of the tumours. Further studies are needed to investigate whether surgery at an earlier stage in the natural history of TART can prevent permanent testicular damage.

Introduction

In adult male patients with congenital adrenal hyperplasia (CAH) testicular adrenal rest tumours (TART) are frequently present with a reported incidence of 50 – 95% (1,2). Because of their location in the mediastinum testis, the tumour can lead to obstruction of seminiferous tubules. In addition to these mechanical effects of the tumour, steroids produced by the tumour may reach the circulation interfering with the secretion of FSH and LH by the pituitary and they may also be toxic to testicular tissue in a paracrine manner, thereby contributing to testicular dysfunction (3-5).

Treatment with high doses of glucocorticoids may lead to suppression of ACTH secretion and reduction of tumour size (6-9). However, high doses of glucocorticoids do not always restore testicular function and may have several side effects (10).

Because of the benign character of the tumours, testis-sparing surgery has been proposed for the treatment of TART. Walker et al. performed testis-sparing surgery in 3 CAH patients (11). Postoperatively, there was good vascular flow and no recurrence of the tumour. Tiryaki et al. reported 2 CAH patients with steroid unresponsive testicular tumours, who were also treated by testis-sparing surgery (12). In both studies no information about pituitary-gonadal function before and after surgery was reported.

We treated 8 adult infertile CAH patients and bilateral TART with testis-sparing surgery. The aim of our study was to evaluate whether testis-sparing enucleation of the tumour can improve pituitary-gonadal function. Here we describe the results of the clinical, biochemical, radiological and histological evaluation of the patients before, and 6 and 22 months after the operation.

Patients and Methods

Patients and surgical procedure

Eight male patients with CAH due to 21-hydroxylase deficiency were selected for operation. Written informed consent was obtained from all patients. All patients had bilateral TART. Five patients had palpable masses (no.1-4, 6). Two patients reported pain and discomfort. Five patients (no.1, 2, 4-6) had been treated with high doses of glucocorticoids in the past to reduce tumour size without success.

The indications for operation are listed in table 1. The age of the patients was 30 ± 8.9 years (mean ± SD; range 23-51 years).
Height SDS was –2.0 ± 1.0 (range -3.5 - -0.2) and BMI was 28.1 ± 4.4 kg/m^2 (range 23.7 – 38.2). Seven patients were Caucasian, one patient (no.8) was of West Indian ethnicity.
Testicular tumour enucleation took place after general or loco-regional anesthesia. The testis, including its tunica vaginalis, was luxated through an inguinal incision and the tunica vaginalis was opened. The testis was incised at the margo anterior testicularis through the testicular tissue until the margin of the tumour was reached. Then a careful blunt dissection of the tumour was undertaken. Finally the tunica albuginea and the tunica vaginalis were closed and the testis was repositioned in the scrotum.

Biochemical analysis

Biochemical analysis was performed in all patients before and after operation. Patient no.1 was operated without complete preoperative hormonal and radiological evaluation. Venous blood was collected from an antecubal vein at 9 a.m. after overnight fasting and before taking the morning medication to measure serum levels of 17-OH progesterone (17OHP), androstenedione (A), testosterone, estrone, ACTH, LH, FSH and inhibin B. The next day at 9 a.m. after overnight fasting blood was collected after taking 8 mg dexamethasone orally at 11 p.m. the evening before to measure serum levels of 17OHP, A, testosterone, estrone and ACTH. The same investigations were performed 6 months after operation in all patients. Twenty-two months after operation again venous blood was collected after overnight fasting and before taking the morning medication to measure the same parameters as described above. Glucocorticoid doses were not changed until completion of the evaluation 6 months after the operation.

Table 1. *Age, phenotype, mutation analysis, height corrected for target height, BMI, daily glucocorticoid and mineralocorticoid therapy at time of operation, and operation indication in 8 male CAH patients with bilateral testicular adrenal rest tumours*

P	Age (years)	Phenotype[a]	Allele 1[b]	Allele 2[b]	Height (SDS)[c]	HSDS-THSDS[c]	BMI (Kg/m^2)	Daily glucocorticoid therapy (mg/m^2)[d]	Daily mineralocorticoid therapy (µg)[e]	Operation indication[f]
1	24	SW	Deletion/ Conversion	Deletion / Conversion	-2.6	-2.8	27.4	32.2 (HC 20-20-20 mg)	400	1,2
2	29	SW	Deletion/ Conversion	Deletion / Conversion	-2.1	-0.7	25.7	16.0 (HC 20-10 mg)	125	1,2
3	23	SW	IVS2-13A/C>G	IVS2-13A/C>G	-1.4	-0.7	25.6	8.2 (HC 8-4 mg, DXM 0.1 mg)	62.5	2
4	32	SW	IVS2-13A/C>G	IVS2-13A/C>G	-1.4	n.a	28.3	16.9 (HC 25-10 mg)	100	2,3
5	26	SW	IVS2-13A/C>G	IVS2-13A/C>G	-1.9	-1.0	38.2	10.8 (HC 10-5-10 mg)	62.5	1,4
6	51	SV	I172N	Deletion / Conversion	-3.1	-2.95	29.0	16.2 (HC 20-10 mg)	-	5
7	31	SW	Deletion/ Conversion	Deletion/ Conversion	-0.2	-1.3	27.0	30.1 (HC 25-40 mg)	125	2
8	26	SW	IVS2-13A/C>G	IVS2-13A/C>G	-3.5	-2.9	23.7	12.1 (DXM 0.5 mg)	62.5	2,3

P = patient number, SDS = standard deviation score, BMI = body mass index, HC = hydrocortisone, DXM = dexamethasone, n.a.= not available. [a]SW = classic salt wasting CAH; SV = classic simple virilising CAH. [b]Nucleotides are numbered according to Higashi's functional CYP21 sequence (19). [c]Height is expressed as SDS and corrected for target height SDS (HSDS-THSDS). [d]Doses of dexamethasone were converted to hydrocortisone equivalents (1 mg dexamethasone = 40 mg hydrocortisone). [e]Mineralocorticoid medication (9-α-fluorohydrocortisone acetate) was taken in one to three doses. [f]1 = poor hormonal control, 2 = infertility, 3 pain/discomfort, 4 = hypogonadotropic hypogonadism, 5 = hypergonadotropic hypogonadism

Hormone assays

Serum testosterone and 17OHP were assessed by ^{3}H-Radioimmunoassay (RIA) after prepurification by means of paper chromatography of ether extracts of the samples, as described previously (13,14). Serum A concentrations in serum and saliva were measured as described earlier (15). Serum estrone was measured by RIA after extraction and Sephadex LH-20 chromatography. The within- and between-assay coefficients of variation were 4.8% and 7.5%, respectively. ACTH was measured by a two-step immunoradiometric assay (IRMA, Dynotest BRAHMS, Berlin, Germany). Serum FSH and LH were determined with a Fluorescence Immuno Enzymatic Assay (Abbott, USA) using a Random Access Analyser (Type AxSYM, Abbott). Dimeric inhibin B was quantified using an ELISA (Oxford Bio-Innovation Ltd, Oxford, UK).

Semen analysis

In 6 of the 8 patients semen analysis was carried out after > 2 days of sexual abstinence before, and 6 and 22 months after operation (16). One patient (no. 5) refused semen analysis. Another patient (no. 6) was sterilized in the past.

Radiological evaluation

All patients underwent testicular MRI before, 6 and 22 months after surgery. All MR studies were performed on a 1.5-T scanner (Magnetom Sonata, Symphony or Avanto, Siemens, Erlangen, Germany), using a body phased-array coil.

Histopathology

All removed tumour tissue was investigated macroscopically and microscopically. Tumours and testis biopsies were fixed in 10% buffered formalin. Tissue sections of 5 µm were cut and stained with haematoxylin & eosin and with Von Giesons elastin stain. Testis biopsies were scored according to Johnsen with a score varying from 0 to 10. A Johnsen score of > 8 is associated with fertility (17).

Results

Radiological evaluation

No apparent residual tumour was seen in any patient on postoperative images. The measured volume of the testicular tumour (mean 9.6 ml, range 0.5 - 29.6) showed a good correlation with the tumour weight ($R^2 = 0.98$). Testicular volumes decreased after surgery in all patients (range -8 to -87%).

Biochemical analysis (table 2)

Before operation, inhibin B levels were significantly decreased in all patients without any correlation with FSH levels. In 4 patients (no. 1,2,4,5) estrone levels were markedly increased with a strong correlation with A levels ($R^2 = 0.9$; $p<0.02$). In three of these patients (no.1, 2, 5) we found suppressed LH and FSH levels suggesting suppression of the hypothalamic-pituitary-gonadal axis due to high serum estrone levels induced by aromatization of A. Despite this we found serum testosterone levels, which were not decreased or were even in the high normal range (no.1). Additionally, testosterone levels decreased after overnight high dose dexamethasone in these three patients (data not shown), indicating that testosterone in blood was mainly derived from conversion of adrenal androgens.

Two patients (no.6 and 7) showed elevated levels of LH and FSH, with in one patient (no.6) a low testosterone concentration. In two patients (no. 3 and 8) LH and FSH and testosterone levels were within the normal range. In these 4 patients (3, 6, 7 and 8) estrone levels were also within the normal range. After operation inhibin B levels remained low in all patients with again no significant correlation with FSH levels. The patients showed a variable increase in FSH and LH levels except in one patient (no.1) who had persistently low LH and FSH levels. Two patients showed a significant decrease in testosterone levels suggesting additional testicular damage due to surgery.

Semen analysis

Before operation azoospermia was found in 5 patients and oligozoospermia in 1 patient. After operation there was no improvement of sperm quality.

Table 2. *Testicular volumes measured by MRI and parameters of pituitary-gonadal axis function and levels of 17-hydroprogesterone (17OHP) and androstenedione (A) before, and 6 and 22 months after testis-sparing surgery in 8 male CAH patients with bilateral testicular adrenal rest tumours*

P		Testicular Volume (ml) [a]			Serum FSH (U/l)[b]			Serum LH (U/l) [c]			Serum Testosterone (nmol/l) [d]			Serum Inhibin B(ng/l)[e]			Serum 17OHP (nmol/l) [f]			Serum A (nmol/l)[g]			Serum Estrone (pmol/l)[h]		
		Pre	Post		Pre	Post		Pre	Post		Pre	Post		Pre	Post		Pre	Post		Pre	Post		Pre	Post	
			6	22		6	22		6	22		6	22		6	22		6	22		6	22		6	22
1	L	nd	nd	1.7	<0.2	<0.2	0.7	<0.2	<0.2	0.1	37.0	23.0	3.6	nd	nd	0	720	160	340	100	74	131	2700	4100	790
	R	nd	nd	1.9																					
2	L	14.2	6.0*	4.9	0.6	12.3	0.7	<0.2	7.1	0.2	14.0	10.0	13.0	76	25	61	480	410	834	86	60	98	1100	1200	1900
	R	17.6	9.9	9.5																					
3	L	8.5	2.9	2.2	8.6	79.2	53.0	5.2	62.6	43.6	17.0	9.5	11.0	65	16	23	26	18	62	1.5	0.74	4.8	140	160	300
	R	8.1	3.4	3.8																					
4	L	8.4	6.4*	6.4	15.9	24.0	24.2	2.9	20.8	17.4	13.0	15.0	12.9	47	19	15	367	470	840	14	23	53.2	650	810	1380
	R	9.7	6.5*	7.3																					
5	L	10.9	5.9	8.0	<0.2	4.9	30.0	<0.2	0.6	10.0	9.8	12.0	11.0	80	17	31	865	630	390	50	52	25	1400	1110	530
	R	11.1	3.5	5.3																					
6	L	6.9	6.3	5.5	55.2	63.3	17.2	44.9	37.2	5.2	7.1	1.3	9.8[i]	10	4	<10	5.1	1.7	2.0	0.9	0.08	2.8	210	230	233
	R	8.4	5.7	4.5																					
7	L	4.5	3.6	4.1	39.3	42.2	44.5	12.3	10.2	19.4	18.0	18.0	10.0	9	17	15	4.3	2.7	12.0	1.2	1.1	0.96	200	160	130
	R	7.0	6.2	5.6																					
8	L	7.6	1.4	1.0	6.3	40.0	<0.2	5.6	28.2	<0.2	20.0	13.0	34.1[i]	5	18	25	10	3.0	195	2.2	0.68	12.6	230	130	284
	R	6.6	4.8	3.4																					

P = patient number, Pre = before operation, Post = 6 and 22 months after operation, nd = not determined, L = left testes, R= right testes, 17OHP = 17-hydroxyprogesterone, A = androstenedione. [a]Testicular volume determined by MRI (*testis showing irregular shaped hyposignal area adjacent to the mediastinum testis after surgery)

Normal values of our laboratory: [b]FSH 1.5 – 11 U/l; [c]LH 1.4 – 8.5 U/l; [d]Testosterone 11 – 45 nmol/l; [e]Inhibin B 150 – 400 ng/l; [f]17OHP 2.0 – 10.8 nmol/l (morning)

[g]A 1.4 – 9.7 nmol/l (morning); [h]Estrone 65 – 220 pmol/l; [i]With suppletion of testosterone

Histopathology

Weight of the tumours in the left testes was 9.3 ± 9.9 grams (mean ± SD; range 1.3–27.4) and in the right testes 8.7 ± 9.1 grams (range 0.45–22.9). Macroscopically, all tumours were firm and multilobular with a yellow to tan color on cut surface and narrow bands of fibrous tissue. Microscopically, the tumours consisted of sheets or confluent cords of large polygonal cells with abundant eosinophilic cytoplasm, separated by dense fibrous tissue strands located in the hilar area of the testis with compression of the rete testis.

The testicular biopsies showed decreased spermatogenesis with reduced Johnsen scores (range 1.0 – 7.6). In all patients focal interstitial fibrosis and peritubular fibrosis was present and in four of the seven patients focal tubular hyalinization was prominent, being most pronounced in patient 6.

Discussion

Our study is the first to provide a complete evaluation of pituitary-gonadal function before and after testis-sparing surgery in CAH patients with TART. All surgical procedures were without complications. Six and twenty-two months after surgery, MRI examination of the testes showed no evidence of residual or recurrent testicular tumour. Complaints of testicular pain and discomfort as reported in two patients disappeared after surgery.

Semen analysis did not improve after surgery with persistently low inhibin B levels in all patients reflecting persistent Sertoli cell dysfunction. As seen in patients 1, 2 and 5, Sertoli cell dysfunction can be masked by simultaneous suppression of FSH secretion due to high serum oestron levels induced by aromatization of adrenal A in these patients. Therefore, inhibin B is a more accurate marker for Sertoli cell function than FSH in CAH patients.

The absence of positive effects on testicular function after operation despite complete removal of the tumours strongly suggests pre-existent irreversible testicular damage in our patients. Indeed, peritubular fibrosis and tubular hyalinization was seen in testes biopsies taken during surgery, which confirms irreversible damage of the testes probably due to longstanding mechanical obstruction in all patients. It is clear, that at this stage surgery can no longer help to restore testicular function.

TART may produce steroids that can contribute to elevated levels of A and 17OHP. Therefore, removal of the testicular tumours may lead to a decrease in the levels of A and 17OHP. However, in our group 17OHP and A levels did not change significantly after

surgery. These observations suggest that surgical treatment is not helpful in improving hormonal control.
Interestingly, all but one of our patients had a homozygous deletion/conversion genotype or a homozygous IVS2-13A/C>G genotype. In an earlier study we showed that in patients who were homozygous or heterozygous for the deletion/conversion mutation tumour size was significantly larger than in patients with other mutations (18). The present study suggests that the IVS2-13A/C>G mutation may also be a risk factor for the development of testicular tumours.

In summary, testis-sparing surgery in CAH patients is a feasible treatment for TART in CAH patients. Complaints of testicular pain and discomfort disappeared after surgery. However, twenty-two months after surgery no improvement in testicular function was seen. Further studies should investigate, if at an earlier stage in the natural history of the testicular adrenal rest tumour testis-sparing surgery might be advantageous.

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Chapter 4

Value of magnetic resonance imaging in the evaluation of testis-sparing surgery in male patients with congenital adrenal hyperplasia and testicular adrenal rest tumours

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Value of magnetic resonance imaging in the evaluation of testis-sparing surgery in male patients with congenital adrenal hyperplasia and testicular adrenal rest tumors

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Submitted

Abstract

Background

Testicular adrenal rest tumours (TART) are a common finding in adult male patients with congenital adrenal hyperplasia (CAH) and may lead to gonadal dysfunction and infertility. The purpose of our study was to assess the value of MRI in calculating the volume of TART and of normal testicular parenchyma before and after testis-sparing surgery.

Patients and methods

Seven adult male CAH patients with bilateral TART underwent MRI before and 6 and 22 months after testis-sparing surgery. Volumes of the tumours and residual testicular tissue were calculated by using the voxel-count and formula-based approximation on T2-weighted MRI. The results of the tumour volume measurements were compared to the weight of the surgical specimen applying linear regression analysis.

Results

The calculated tumour volumes measured by voxel-count method showed a stronger linear correlation with the weight of the tumours (R^2=0.99, p< 0.001) than calculated by formula-based approximation (R^2 =0.95, p< 0.001) (p=0.02). No residual tumour tissue was seen on postoperative images. In all patients residual testicular tissue significantly decreased in volume (mean; -41 %, range; -9 – -81, p<0.001).

Conclusion

Testicular MRI can provide accurate volume assessment of TART by using the voxel-count method. This method can therefore be used for monitoring and follow up of TART during medical treatment or after surgery. Furthermore, MRI is a good method for measuring the volume of normal testicular tissue and may be used as an additional marker in the evaluation of gonadal function.

Introduction

Testicular adrenal rest tumours (TART) are a common finding in adult male patients with congenital adrenal hyperplasia (CAH) (1, 2). The tumours are almost always bilaterally present and have no malignant features, however because of their location in the rete testis they can lead to obstruction of the seminiferous tubules with irreversible damage of testicular parenchyma. Therefore, detection and treatment of the tumours is an important goal to prevent gonadal dysfunction. It is thought that the tumours consist of adrenal-like tissue and that increased plasma levels of ACTH may induce tumour growth. Therefore, the first choice of treatment is optimizing glucocorticoid therapy to suppress ACTH levels and consequently to diminish tumour size. Since, several patients do not respond to medical treatment testis-sparing surgery has been introduced in an attempt to improve obstruction of seminiferous tubules and gonadal function. Two studies describe testis-sparing surgery in 5 patients (3, 4). However, these studies did not mention whether complete removal of the tumours was achieved and whether normal testicular tissue was preserved after surgery.

Exact measurement of tumour size is of great importance for the monitoring of CAH patients with TART who are treated with increased doses of glucocorticoids as well as for preoperative evaluation in the case of steroid unresponsiveness. Measurement of the volume of normal testicular parenchyma may be a useful tool to evaluate gonadal function as testicular volume correlates with sperm count and fertility (5).

Both magnetic resonance imaging (MRI) and ultrasound are sensitive methods to detect small non-palpable testicular tumours (6-8). Furthermore, both of them are also useful for measuring tissue volumes in vivo. Ultrasound (US) can, however, provide only the total volume of the testis (i.e., the sum of normal testicular tissue and tumour tissue) because the volume is estimated with a formula by measuring transverse and longitudinal diameters of each object (9, 10). In CAH patients with TART calculation of the normal testicular volume is not possible by ultrasound since both the volumes of both the entire testis and the tumour need to be measured separately to calculate the volume of the normal testicular tissue. The voxel-count method with MR images, i.e. using the sum of all voxel volume lying within the boundaries, however, can accurately measure the volume of the testes irrespective of its shape (11-16). Thus, this method is expected to provide accurate assessment of the volumes of TART as well as of normal testicular tissue in patients with TART.

We studied seven adult CAH patients with longstanding bilateral TART and gonadal dysfunction who were treated with testis-sparing surgery in our institution (17). The aim of

our study was to assess the value of MRI in calculating the volume of TART before surgery by comparing two different methods i.e. the voxel-count method and the formula-based approximation on MR images. The results of both methods were related to the weight of the surgical specimen after surgery. Furthermore, we evaluated the value of MRI in detecting residual tumour tissue after surgery and in calculating the volume of the normal testicular parenchyma before and after surgery both by using the voxel-count method.

Patients and Methods

Patients

In seven male CAH patients (mean age: 31 years, age range: 23–51 years) with bilateral TART, testis-sparing surgery was performed because of unsuccessful glucocorticoid therapy (*n*=5), infertility (*n*=5), poor hormonal control despite rigorous treatment (*n*=2), pain or discomfort (*n*=2), and/or hypogonadism (*n*=2). Six tumours in three patients were palpable at physical examination. The diagnosis of CAH due to 21-hydroxylase deficiency was confirmed by mutation analysis in all patients. All but one patient had the classic salt-wasting form of CAH, which was treated with glucocorticoids and mineralocorticoids since the neonatal period. One patient had the simple virilising form of CAH diagnosed at the age of five years. The results of endocrine and clinical investigations in these patients have been reported elsewhere (17). Local ethical committee approval was not required for the retrospective evaluation of clinical data.

MR imaging

All patients underwent testicular MRI before, and 6 and 22 months after surgery. All MR studies were performed with a 1.5-T scanner (Magnetom Symphony, Sonata, or Avanto, Siemens Medical Solutions, Erlangen, Germany) using a CP-spine array coil or a matrix array spine coil in the prone position. After obtaining localizing images, three sets of orthogonal T2-weighted images were obtained with turbo spin echo (TSE) sequence with TR/TE, 4,300–6,730/135; flip angle, 150°; echo train, 19; 280 × 320 pixel; field of view, 150 mm, or true fast imaging with steady state precession (FISP) sequence with TR/TE, 4.3–4.5/2.2; flip angle, 80°; matrix, 192–256 × 256; field of view, 131–175 × 175 mm. Transverse T2-

weighted images were obtained using TSE sequence in all patients, while sagittal and coronal images were acquired with TSE or true FISP sequence. In all but two examinations, a slice thickness of 2.5 mm with 10% (0.25 mm) intersection gap was used for transverse TSE images, while a thicker slice of 3.0 or 4.0 mm with 10% gap was applied in two preoperative examinations with large tumours and true FISP images.

Image evaluation

The volume of each testis and tumour was calculated by a single radiologist (S.T.), who was blinded to the clinical data and surgical records of the patients, at a personal computer (Power Mac G5; Apple Computer, Cupertino, CA) using two different methods, namely voxel-count method and formula-based approximation.

In voxel-count method a segmentation line was outlined for the whole testis including both the normal testicular tissue and the tumour on each section of transverse T2-weighted images using a *Java* based public domain software (*ImageJ*, version 1.34s; National Institutes of Health, Bethesda, MD). The segmentation line was drawn at the middle point of the change in signal intensity for avoiding partial volume averaging. Subsequently, the sum of all areas of the segmented sections was multiplied by the section thickness including intersection gap to calculate the volume of each object. As TART is located adjacent to the mediastinum testis showing a low signal intensity on T2-weighted images (6,8), a localized low-signal-intensity area within the testis was regarded as TART and traced in the same way. In postoperative follow-up the entire low-signal-intensity area on T2-weighted images adjacent to the mediastinum testis was traced, because postoperative scar tissue can also show low signal intensity, which might be indistinguishable from low-signal-intensity TART. Accordingly, the volume of the tumour and/or postoperative scar, as well as whole testis, was calculated in each examination.

In formula-based approximation, which is frequently applied to US (10), the long and short axis of each testis were measured on the open-source DICOM image processing software (OsiriX2.4: downloaded at *http://homepage.mac.com/rossetantoine/OsiriX*). The whole testicular volume was calculated by using the empiric formula of *Lambert* (18): volume = length × width × thickness × 0.71, while the volume of tumour was calculated by using the ellipsoid formula: volume = length × width × thickness × π/6. In postoperative follow-up the volume of low-signal-intensity area could not be evaluated with a formula because of its irregular shape. In each method all measurements were repeated twice for a set of MR images

with at least 4 weeks interval. Then, the average of two measurements was used for the analysis. The volume of normal testicular tissue was calculated by subtracting the tumour volume from the whole testis, which was only available for the voxel-count method. Furthermore, the shapes of low-signal-intensity area and signal intensity of the normal testicular tissue, as well as the presence of residual tumour in postoperative examinations were evaluated.

Statistical Analysis

The measured volume of TART was compared to the weight of surgical specimen. Linear regression analysis was used for evaluating the relationship between the measured volume and the weight, with the weight of surgical specimen as the independent variable and the measured volume as the dependent variable. The strength of the linear association for each method was tested by using Fisher *z*-transformation. Bland and Altman analysis was performed to assess the intraobserver variation of each measurement, calculating the differences between the first and second measurement, mean difference, and standard deviation (SD) of the difference (11, 19). As the SD of the difference is a measurement of variation, larger SD of the differences indicates poorer repeatability of the method.
One-way repeated measures analysis of variance (ANOVA) was used to determine significant changes of the volume of normal testicular tissue that was calculated by subtracting the volume of tumour or low-signal-intensity area from the whole testis volume with voxel-count method. Tukey's post hoc multiple comparisons were conducted among the groups with overall significant probability value of ANOVA. Statistical analyses were performed by using computer software package (GraphPad Prism for Macintosh 4.0c; GraphPad Software, San Diego, CA) and a two-tailed *p*- value of less than 0.05 was considered statistically significant.

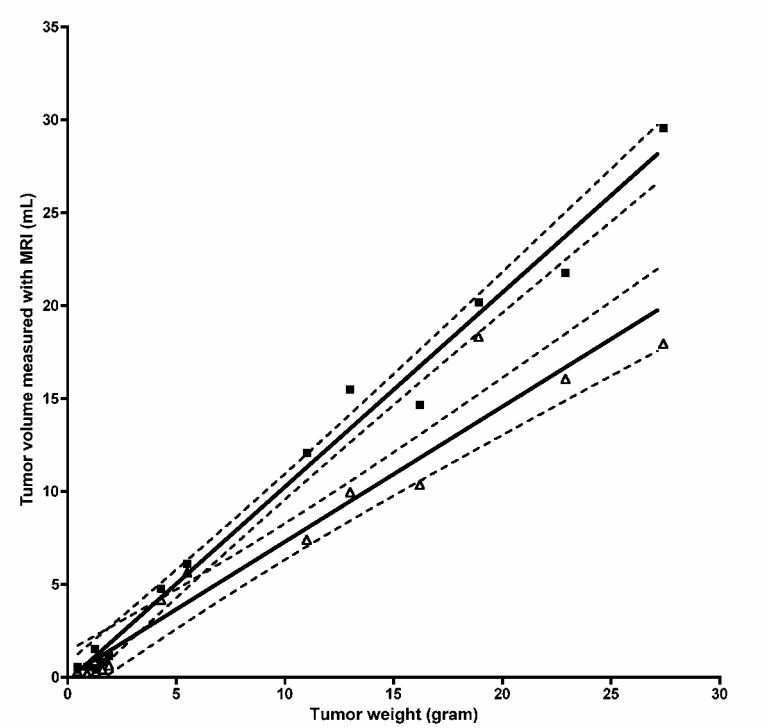
Results

Assessment of tumour size before surgery and of residual tumour after surgery

TART, weighted 9.0 ± 9.2 g (mean ± SD; range 0.45 to 27.4), were enucleated in all patients without complications. The tumour volumes measured by both voxel-count method and ellipsoid formula showed a strong linear relationship with the weight of the tumour (linear

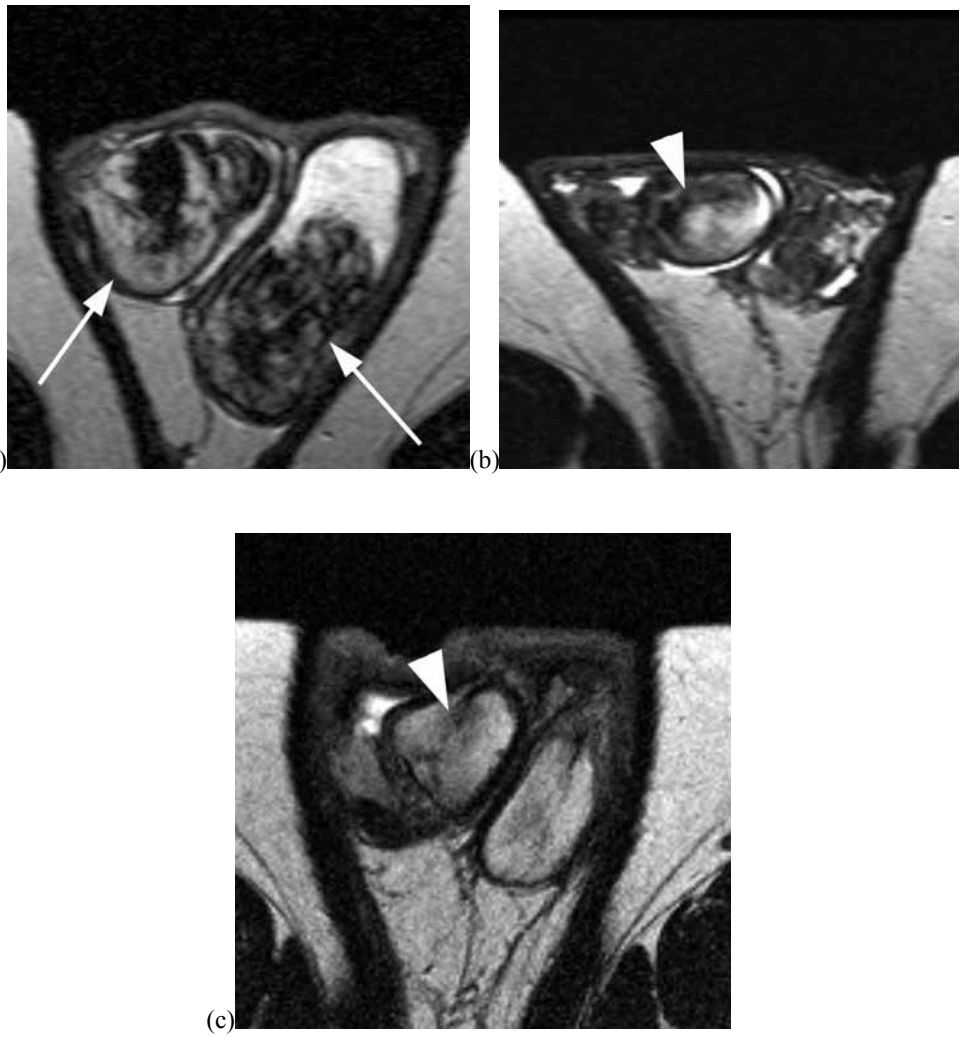
regression analysis $R^2 = 0.99$; $p < 0.001$ and $R^2 = 0.95$; $p < 0.001$, respectively), with voxel-count method showing a stronger relationship than formula-based method (Fisher *z*-transformation; $p = 0.02$) (Figure 1). The volumes of the tumours calculated by the voxel-count method (9.3 ± 9.7 ml; range 0.5 to 30) were significantly larger than using ellipsoid formula (6.6 ± 6.9 ml; range 0.19 to 18.3) (paired *t* test; $p = 0.01$). No residual masses or nodules were observed on postoperative MRI. In two patients with larger tumours (11.0 to 27.4 g) irregular low-signal-intensity areas radiating from the mediastinum testis were observed 6 months after surgery, but decreased in size at 22 months follow-up (Figure 2).

Figure 1. *Comparison of tumor weight of surgical specimen and calculated tumor volume with voxel-count method (■) and with ellipsoid formula (□).*



The solid line indicates the linear relationship obtained from liner regression analysis for each measurement, while the set of curved dashed line represent the 95 % confidence bands. The volumes of the tumours calculated by the voxel-count method were significantly larger than those approximated by the formula-based aproximation, although both showed a strong linear relationship with the weight of tumours (linear regression analysis $R^2 = 0.99$, $p < .001$, $R^2 = 0.95$, $p < .001$, respectively).

Figure 2. *Pre- and postoperative appearance of the testes of a 53-year old CAH patient with bilateral TART on T2-seighted TSE images (4,700 ~ 5,340 / 135).*



(a) On preoperative images, heterogeneous low-signal-intensity tumors (arrows) were displacing surrounding high signal normal testicular tissue.

(b) A low-signal-intensity area radiating from the thickened mediastinum testis (arrowhead) was seen on postoperative images obtained 6 months after successful tumor enucleation.

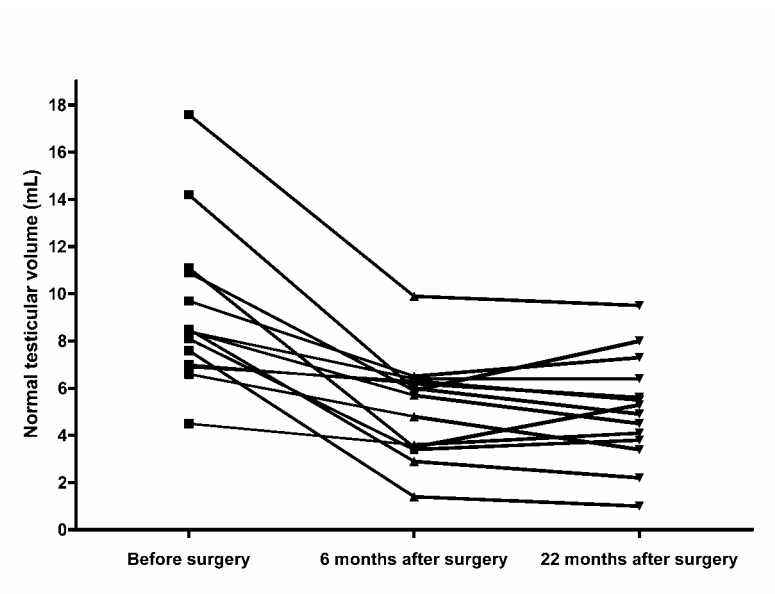
(c) Twenty-two months after surgery the low-signal-intensity area decreased in size (arrowhead).

Note: All images are displayed with the same FOV and magnification.

Assessment of the volume of normal testicular parenchyma before and after surgery

Figure 3 demonstrates the changes of normal testicular tissue before and after testis-sparing surgery in 14 testes of 7 patients. All residual testicular tissue decreased in volume 6 months after surgery (-41 ± 23%; range -9 to -81%) without further changes 22 months after surgery (-43 ± 23%; range -8 to -87%) (Tukey's multiple comparison test; $p < .001$). The mean total normal testicular volume (i.e., sum of the normal testicular volumes of both testes) before surgery was 18.5 ± 6.7 ml (range; 11.5 to 31.8 ml) and decreased to 10.2 ± 3.9 ml (range; 4.4 to 14.4 ml) 22 months after surgery.

Figure 3. *Changes of residual normal testicular tissue in 14 testes of 7 patients. All residual tissue decreased in volume 6 months after surgery (-41 ± 23%; range -9 to -81%) without further changes 22 months after surgery measured by voxel-count method.*



Intraobserver variation of measurement

The intraobserver variation of each measurement is shown in Table 1. The SDs of the difference between the first and second measurement, representing intraobserver variation, were limited to 12.7% of the tumour volume and to 10.0% of the whole testicular volume with the voxel-count method, while they were limited to 38.1% and 25.0% with the formula-based method.

Table 1 *Difference between the first and second measurement of the tumour and the whole testicular volume in preoperative MRI with two different measurements.*

	Mean Difference (ml)	SD of the Difference (ml)		Relative SD of the Difference (%)	
Tumour volume					
Voxel-count method	-0.7	0.8	(-2.3, 0.9)	12.7	(-36.3, 13.6)
Formula-based method	1.44	2.4	(-3.2, 6.1)	38.1	(-54.0, 95.2)
Whole testicular volume					
Voxel-count method	-1.1	1.2	(-3.5, 1.2)	10.0	(-31.1, 9.1)
Formula-based method	-3.8	4.2	(-12.1, 4.4)	25.0	(-79.8, 18.0)

Values in parentheses are 95% limits of agreement. Relative SD of the difference are shown as the percentage of the tumour or whole testicular volume.

Discussion

Our study demonstrates that the volume of TART in CAH patients can be exactly measured with MR imaging, showing a strong linear relationship with the true tumour weight with the voxel-count method being more accurate than the formula based method. To our knowledge, this is the first study comparing TART volume measured with MR imaging and the weight of the surgical specimen. In the literature, several formulas, such as the ellipsoid, the prolate spheroid, or the empiric formula of *Lambert* (18), have been proposed for calculating testicular volume based on US measurement of testicular length, width, and height. However, these formulae can provide only the total volume of the testis in theory, because there is no appropriate formula for estimating a volume of irregular shaped tumour. Voxel-count method, the sum of all voxel volume lying within the boundaries, has shown high accuracy and repeatability for volume measurement in various regions [11-16]. The technique is, however, hardly applicable to US because of inconsistent voxel volume of each ultrasound image. Therefore, we applied MR imaging for tumour volume measurement instead of US, which is the widely used technique to assess testicular volume in situ.

The accuracy of the voxel-count method depends on the spatial resolution or the volume of each voxel (12). Our scanning parameter of T2-weighted images achieved in-plane resolution of 0.47 × 0.47 mm with a section thickness of 2.75 mm. The parameter yielded a voxel volume of 0.60 mm^3 (μl), which should be sufficient to measure the mean tumour volume of 9.3 ml (ranged 0.5 to 29.6 ml). Indeed, the volume measurement with voxel-count method showed a very good relationship with the weight of the tumour (R^2 = 0.99), with less intraobserver variation than the formula-based method. As the level of hormonal control

cannot predict the response of tumour growth to glucocorticoid therapy (20), exact measurement of tumour size is important for monitoring TART in CAH patients who are treated with increased doses of glucocorticoids, as well as for preoperative evaluation for steroid unresponsive TART. Therefore, MR imaging with voxel-count measurement is advised in evaluation of tumour volume in CAH patients with TART. In two patients with large TART low-signal-intensity areas were seen on MRI 6 month after surgery. Although these lesions showed similar signal intensity as TART, they were distinguishable from residual tumours because of their irregularly radiating shapes, indicating scar tissue. No residual tumour was confirmed by follow-up MRI, as all these low-signal-intensity lesions decreased in size in 22 months after surgery without additional treatments. Thus, no residual tumour was found by MRI, suggesting that TART can be successfully removed by testis-sparing surgery.

Our study revealed that MR imaging using voxel-count method is also useful in evaluating testicular parenchyma in CAH patients with TART. As mentioned earlier accurate determination of testicular volume is of importance in evaluating the status of spermatogenesis, as testicular volume strongly correlates with sperm count and fertility. Arai et al. reported that patients with a total testicular volume of less than 10 ml were azoospermic, while volumes of 10-20 ml were associated with severe oligozoospermia (5). The mean total testicular volume in our patients was 18.5 ml before surgery, and none of our patients had a total testicular volume of 15 ml or larger after surgery. Furthermore, we detected a decrease in normal testicular tissue volume after the enucleation of TART in our group of CAH patients with longstanding tumours. It is known, that the presence of long-standing tumours may lead to irreversible damage of the normal testicular tissue due to chronic obstruction of the seminiferous tubules. Testicular biopsies taken during testis-sparing surgery demonstrated peritubular fibrosis and tubular hyalinization in our group of patients, which confirmed irreversible damage of the testes (21). However, probably testis-sparing surgery may lead to additional damage of the testicular tissue with consequently a decrease of the normal volume of testicular parenchyma. Therefore, further studies are needed to evaluate the the benefits of testis-sparing surgery in CAH patients with TART.

Our study has some limitations. First, only seven CAH patients with TART were described. However, previous studies were case reports or smaller patient series (3, 4, 22, 23). To our knowledge, our study is the largest reported series of male CAH patients with TART treated with testis-sparing surgery. Second, we compared the tumour volume calculated with MRI to the weight of surgical specimen, instead of the volume. As the enucleated tumours were

generally small and sometimes fractionated, volume measurement of specimen would be far less accurate than weight measurement. Thus, we believe that the weight of surgical specimens is more accurate for comparison than the tumour volume.

In conclusion, in male CAH patients with TART testicular MRI with the voxel-count method is useful for accurate volume assessment of TART and can therefore be used for monitoring and follow up of tumour size during medical treatment and before surgery and also for detection of residual tumour after testis-sparing surgery. Furthermore, MRI is a good method for measuring the volume of normal testicular tissue and may be used as an additional marker in the evaluation of gonadal function.

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Chapter 5

Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21- hydroxylase deficiency

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Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21- hydroxylase deficiency

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Abstract

Objective

Testicular adrenal rest tumours (TART) are a well-known complication in adult male patients with congenital adrenal hyperplasia (CAH) with a reported prevalence of up to 94%. In adulthood the tumours are associated with gonadal dysfunction most probably due to longstanding obstruction of the seminiferous tubules. Aim of our study was to determine the presence of TART and their influence on gonadal function in childhood.

Design

Retrospective study.

Patients and methods

Scrotal ultrasound was performed in 34 children with CAH due to 21-hydroxylase deficiency who were between 2 and 18 years old. FSH, LH, testosterone and inhibin B concentrations were measured in serum of 27 patients.

Results

TART were detected by ultrasound in 8 of 34 (24%) children. In two of them bilateral tumours were found. All lesions were located in the rete testis. Seven patients had the salt wasting type of CAH, one patient had the simple virilising type of CAH. Mean tumour size was 4.1 mm (range 2 – 8 mm). In none of the patients tumours were palpable. Two children with TART were between 5 and 10 years old, the other six children were above 10 years old. In all children with TART LH, FSH, testosterone and inhibin B levels were similar to in patients without TART.

Conclusion

TART can be found in CAH children before the age of 10 years. The absence of gonadal dysfunction in our group of children suggests that gonadal dysfunction as frequently reported in adult CAH patients with TART develops after childhood.

Introduction

Testicular adrenal rest tumours (TART) are frequently found in adult males with congenital adrenal hyperplasia (CAH) with a reported prevalence of up to 94% (1). The tumours are always benign. However, because of their typical location within the rete testis the tumours may lead to obstruction of the seminiferous tubules with gonadal dysfunction and infertility (2). Therefore, it may be important to detect and treat the tumours in an early stage. Both ultrasound and MRI are good imaging modalities for the detection and follow up of TART, especially when they are not palpable on physical examination (3). The presence of TART in children is described mostly in case reports (4-7) and only a limited number of studies describe its prevalence in larger populations of children and adults (8-10). Avila et al. detected TART by ultrasound in 8 of 38 male CAH patients (age 6 – 31 years) (9). The mean age of the patients was 14.8 years and 7 of the 8 patients with TART were below 18 years old. The youngest patient was 6.2 years old. The total number of investigated patients below 18 years was not reported. Vanzulli et al. described a prevalence of 27% of TART in a group of 30 CAH patients between 9 and 32 years (10). In the 24 investigated patients below 18 years 7 (29%) had TART. However, these studies did not focus on childhood age and did not present information on gonadal function. It is suggested that adrenal rest cells in the testes are already present in the embryological period but the onset of tumour growth is not known. Shanklin et al. studied autopsy material of patients with CAH and detected TART in 3 of 7 patients less than 8 weeks old (11). Other studies could not detect testicular tumours by ultrasound before the age of 10 (12). The aim of our retrospective study was to investigate the incidence of TART in our group of male CAH children. Furthermore, we measured FSH, LH, testosterone and inhibin B levels in this group of CAH children in order to detect possible negative effects of these tumours on gonadal function in childhood. We show that TART are already present in childhood and that in our study group gonadal function is not disturbed.

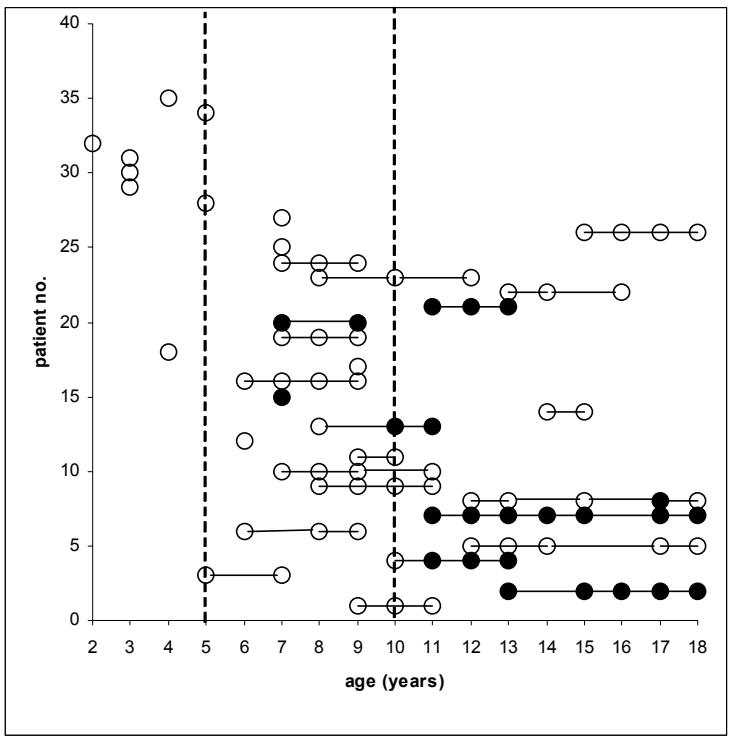
Patients and methods

Patients

Scrotal ultrasound was performed in all 34 male children with CAH due to 21-hydroxylase deficiency who were regularly presented at our outpatient pediatric endocrine unit.

The patients were ranged in 3 age categories: 1 to 5 years, 5 to 10 years, 10 to 18 years old. The mean age at the first ultrasound was 8;1 (years;months) (range 2;7 to 15;0). In six children the first scrotal ultrasound was performed before the age of 5 years, in 19 children between 5 and 10 years and in 9 children between 10 and 18 years. Ultrasound scanning was repeated in intervals of one to two years in most patients (figure 1).

***Figure 1**. Overview of all scrotal ultrasound investigations in 34 male children with congenital adrenal hyperplasia aged 2 a 18 years. Each circle represents one ultrasound investigation. Open circle = no TART detected; black circles = TART detected.*



In all patients the diagnosis CAH was made on symptoms and laboratory tests and was confirmed by mutation analysis. Twenty-four patients had the classical salt wasting (SW) type, eight patients had the simple virilising (SV) type and two patients had the late onset (LO) type of CAH. All SW patients were diagnosed in the first months of life with exception of patient six who had uretral valves. He was falsely diagnosed as having pseudohypoaldosteronism and was treated only with salt suppletion in the first year of life. The diagnosis CAH in this patient was made not before the age of 1;7 years. All patients were

treated with hydrocortisone three times a day and in the case of SW also with fludrocortisone. During follow-up, therapy was monitored each 3 months by evaluating clinical symptoms, anthropometrical measurements (length and weight) and biochemical measurements. Glucocorticoid dosing was monitored by determination of 17-hydroxyprogesterone (17OHP) and androstenedione (A) levels in peripheral blood collected in the morning before taking the glucocorticoid medication in children below 4 – 5 years old and by measuring 17OHP and A in three saliva samples collected before each of three gifts glucocorticoid medication in older children. Undertreatment was defined as a morning androstenedione concentration before taking the first glucocorticoid dose above the morning reference range for pubertal stage; overtreatment was defined as a morning androstenedione concentration below the morning reference range for pubertal stage (13).

For evaluation of the pubertal status the Tanner stages were used (14). Bone maturation was determined according to Greulich and Pyle (15) in order to determine the hormonal control over years.

Radiological evaluation

All ultrasonographic evaluations were performed by one of the three staff pediatric radiologists in our centre with extensive experience in scrotal ultrasound.

The imaging was done on a Philips (ATL, Bothell, WA, USA) model (500hdi) with multi-probe capabilities (4.5-11 mHz) and the lesions were measured by caliper. At least 3 transverse and axial images of each testis were made, with at least one Doppler image containing the lesion(s). Testicular tumours within the mediastinum testis can be detected by ultrasound when they are at least 2 mm (3).

Hormone analysis

LH, FSH, testosterone and inhibin B concentrations in serum were measured in patients above the age of 5 years old. We measured LH and FSH concentrations in 27 children, testosterone concentrations in 26 children and inhibin B concentrations in 23 children. LH and FSH were determined with a Fluorescence Immuno Enzymatic Assay (Abbott, USA) using a Random Access Analyser (Type AxSYM, Abbott). Testosterone was assessed by RIA after prepurification by means of paper chromatography of ether extracts of the samples, including correction for procedural losses, as described previously (16,17).

Dimeric inhibin B was quantified using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) specific for measurement of inhibin B in human serum (Oxford Bio-Innovation Ltd, Oxford, UK). The assay was performed according to the manufacturer's instructions.

Results

Radiological evaluation

Figure 1 summarizes the age of the first scrotal ultrasound and the frequency of follow-up in all 34 investigated children. TART were detected by ultrasound in 8 of the 34 investigated children (24%). In two of them bilateral tumours were found (no.1 and 8). The patients' characteristics are listed in table 1. Seven patients had the SW type and one patient had the SV type of CAH. In none of the patients tumours were palpable on physical examination. In none of the children in the group below 5 years TART were detected. In two children in the group between 5 and 10 years (10% in this age group) and in six children in the group between 10 and 18 years old TART were detected (66% in this age group). The mean tumour size was 4.1 mm (range 2 – 8 mm). Two children (no. 1 and 2) showed progression of the tumour size on follow- up ultrasound, one child (no.7) showed regression of tumour size. All lesions were located near the mediastinum testis and were described as hypoechoic round lesions (fig. 2), occasionally with an echogenic focus within (fig. 3).

Table 1. *Mutation analysis, phenotype, age at diagnosis CAH, age at first ultrasound, age at first detection of TART and tumour size at first detection in eight CAH children with TART*

P	Allele 1[a]	Allele 2[a]	Phenotype[b]	Age at diagnosis of CAH (Year;months)	Age at first ultrasound (Years;Months)	Age at diagnosis of TART (Years;Months)	Tumour size at first detection
1	Del/Conv	329-336 del	SW	Neonatal	13;10	13;10	L3 mm R 3 mm
2	Del/Conv	c.1066C>T	SW	Neonatal	11;9	11;9	L 2 mm R -
3	Del/Conv	c.841G>T, c.1066C>T c.952C>T	SW	Neonatal	8;1	10;1	L 4x2 mm R -
4	IVS 2-13 C.841G>T	c.952C>T	SW	0;3	7;3	7;3	L 5 mm R-
5	Del/Conv	Del/Conv	SW	Neonatal	7;7	7;7	L - R 3 mm
6	c.1066C>T	c.1066C>T	SW	1;7	11;3	11;3	L - R 3 mm
7	Del/Conv	c.707 T>A c.710 T>A c.716 T>A	SW	Neonatal	12;2	17;0	Li - Re 4 mm
8	c.515T>A	c.707 T>A c.710 T>A c.716 T>A	SV	3;4	10;7	11;9	L 6 mm R 8 mm

P = patient number, [a]*Nucleotides are numbered according to Higashi's functional CYP21 sequence (26)*

[b]*SW = classic salt wasting CAH; SV = classic simple virilising CAH*

Figure 2. *Scrotal ultrasound of patient 2 at the age of 13 years. Transverse image shows a mostly hypoechogenic rounded lesion in the left testis (arrow) near the rete testis (broken arrow).*

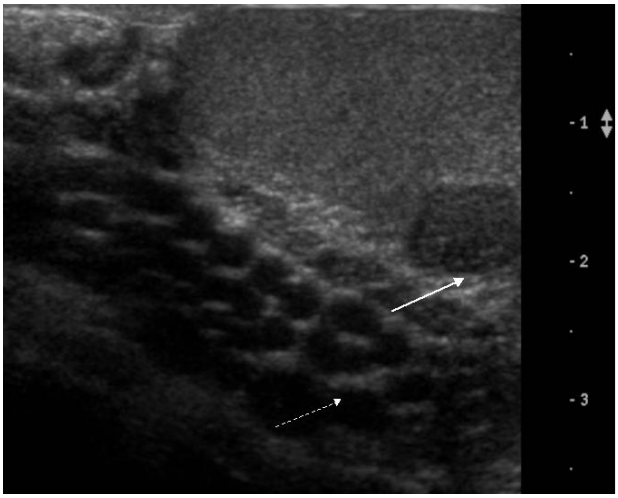
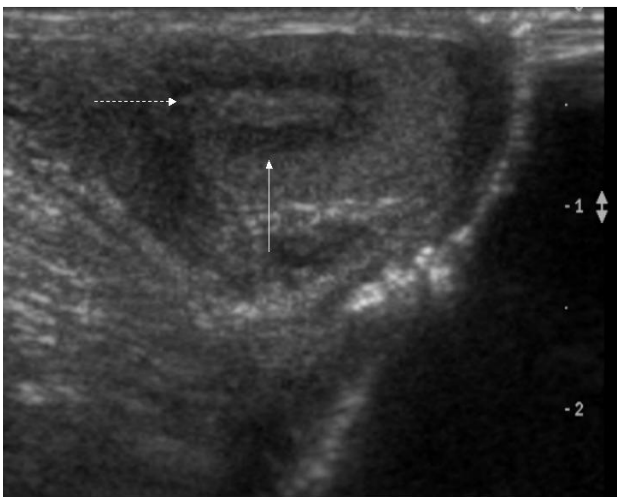


Figure 3. *Scrotal ultrasound of patient 6 at the age of 18 years showing a hypoechoic lesion (arrow) with an echogenic centre (broken arrow).*

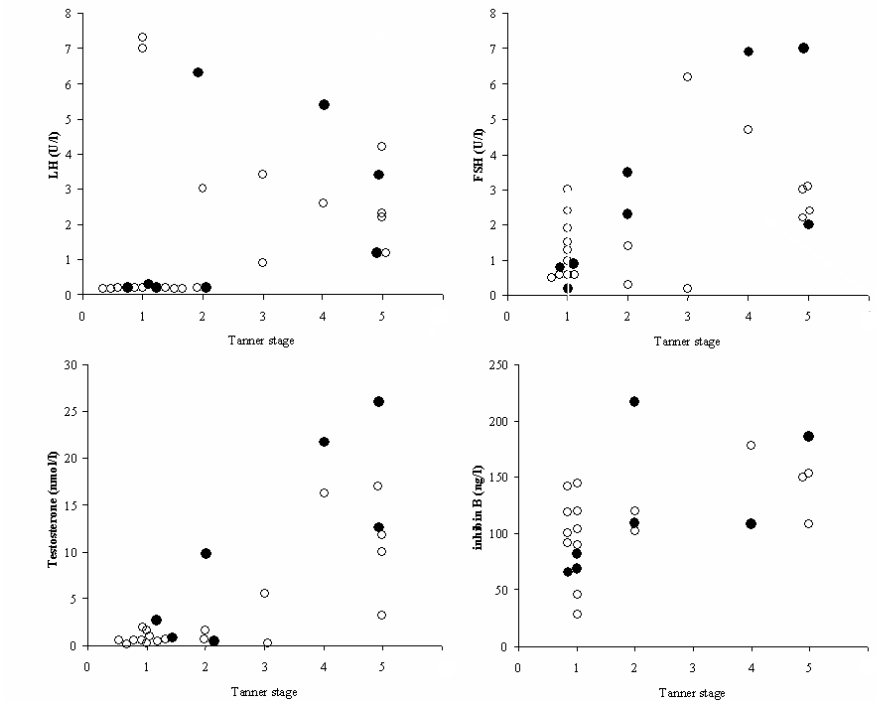


Gonadal function

At the time of biochemical evaluation three patients (no. 1, 2 and 7) had (nearly) completed puberty (Tanner stage IV and V). Two other patients (no. 3 and 8) showed no signs of puberty (Tanner stage I). In three patients (no. 4-6) puberty had started (Tanner stage II). In all 27 children in whom biochemical analysis was performed LH, FSH, testosterone and inhibin B concentrations were within the normal range for pubertal stage, with the exception of 2 CAH

children without TART (age 8 and 7 years old) with isolated increased LH levels. Precocious puberty in these patients was excluded. There was no clear difference in laboratory values between children with and without TART (fig. 4).

Figure 4. *Serum levels of LH, FSH, testosterone and inhibin B in male CAH children without TART (open circles) and with TART(closed circles) related to pubertal stages 1 – 5 according to Tanner(14)*



Normal values of LH (U/l) depending on pubertal stage(I – V): median (5 – 96 percentiles) (ref. 27)
I: 0.08 (< 0.05 – 0.99) II: 0.88 (0.11 – 2.97) III: 2.03 (0.51 – 5.42) IV: 2.89 (1.11 – 5.89) V: 3.4 (1.53 – 6.33)
Normal values of FSH (U/l) depending on pubertal stage: median (5 – 96 percentiles) (ref. 27)
I: 0.85 (0.25 – 2.55) II: 1.95 (0.07 – 4.39) III: 3.5 (0.94 – 9.68) IV: 3.61(1.98 – 6.88) V: 3.1 (1.38 – 7.52)
Normal values of testosterone (nmol/l) depending on pubertal stage: median (5 – 96 percentiles) (ref. 27)
I: < 0.2 (< 0.2 – 0.9) II: 1.9 (< 0.2 – 13.4) III: 8.4 (0.9 – 21.2) IV: 17.2 (7.7 – 26.5) V: 21.0 (11.3 – 32.3)
Normal values of inhibin B (ng/l) depending on pubertal stage: median (5 – 96 percentiles) (ref. 27)
I: 78 (35 – 182) II: 195 (62 – 338), III 163 (78 – 323), IV 188 (67 – 304), V: 187 (95 – 323)

Hormonal control

Six of the eight children with TART had a bone age similar to calendar age. Two children showed advanced bone age of maximally 2 years (no. 3 and 5). Five of the eight children with TART were overtreated at the time of TART diagnosis. Therefore, in these patients the

glucocorticoid medication was not increased when TART was detected. One patient (no.2) was adequately treated. Also in this patient glucocorticoid medication was not increased because of the suppressed salivary androstenedione levels during the rest of the day. Two patients (no. 1 and 7) were undertreated at the time of detection of TART (table 2): Patient 1 was treated with adequate doses of hydrocortisone but there was lack of compliance. After TART detection the glucocorticoid doses was increased without any effect on tumour size and with persistently poor compliance until the age of 18 years. In patient 7 the glucocorticoid dose was increased after detection of TART. Six months later the androstenedione levels were suppressed and the tumour was no longer detectable on ultrasound.

Table 2. *Salivary levels of androstenedione in eight male children at the time of TART detection.*

P	Pubertal stage[a]	A[b] Morning (nmol/l)	A[b] Noon (nmol/l)	A[b] Evening (nmol/l)	Treatment[c]
1	V	0.94	0.65	0.5	Undertreatment
2	V	0.25	0.1	0.08	Adequate treatment
3	I	0.14	< 0.06	0.04	Overtreatment
4	I	< 0.02	< 0.02	< 0.02	Overtreatment
5	I	0.07	< 0.03	< 0.036	Overtreatment
6	I	0.08	0.04	< 0.04	Overtreatment
7	IV	2.7	1.6	0.62	Undertreatment
8	I	0.061	0.03	< 0.02	Overtreatment

P = patient number, A = salivary androstenedione levels. Salivary samples were collected three times a day before taking the glucocorticoid medication (morning between 7.00 – 9.00a.m; noon between 13.00 and 15.00 p.m; evening between 21.00 and 23.00 p.m). [a] Pubertal stage according to Tanner (14) [b]Normal salivary androstenedione values : Tanner I: 0.02 – 0.25 nmol/l. Tanner II – V: 0.14 – 0.63 nmol/l (13) [c]For definitions of undertreatment, overtreatment and aequate treatment see text.

Discussion

The present study shows that in male CAH children TART are already present in childhood with a prevalence of 24%. This finding is in agreement with previous studies (9,10). However, our study is the first focusing exclusively on childhood age in a larger patient group and gives additional information about gonadal function of CAH children with and without TART.

None of the tumours were detectable by palpation and none of the children with testicular tumours showed signs of gonadal dysfunction.

The etiology and pathogenesis of TART in CAH patients is not completely understood. Several studies documented the production of adrenal specific steroids or the presence of adrenal specific enzymes in these tumours (18,19). Therefore, TART are thought to arise from aberrant adrenal cells in the testes. In the embryological period cells destined to become adrenal or gonadal cells derive from neighboring areas of the coelomic epithelium and are morphologically identical (20). During further development a limited number of "adrenal" cells may migrate together with the descending testis. Aberrant adrenal tissue within the testes is reported with an incidence of 7.5 to 15% (autopsy and surgical findings) in healthy neonates and normally regresses in early infancy (21). Our data suggest that the incidence of aberrant adrenal tissue in the testes is probably underestimated because under unstimulated conditions aberrant adrenal tissue is difficult to detect. In CAH patients aberrant adrenal tissue may grow due to chronically elevated levels of ACTH or other unknown growth promoting factors and may explain the increase in tumour detection by ultrasound during childhood.

The youngest CAH patient with TART reported in the literature was only several weeks old (11). Recently, we detected a small testicular tumour at post mortem histopathological examination in a 2-year-old male CAH patient who died in an Addisonian crisis (unpublished data). The youngest patient with TART in our present study was 7 years old. Therefore, it can be speculated that tumour growth starts during childhood or even in prenatal life.

Until now the exact mechanisms responsible for tumour growth are uncertain. ACTH may be an important stimulator of tumour growth because ACTH receptors are present on the tumour tissue and some authors describe a relation between poor hormonal control and progression of tumour growth (22,23). Although adequacy of substitution therapy was not systematically assessed in our patients none of our patients had documented periods of poor hormonal control in early childhood or puberty. The absence of advanced skeletal maturation in most patients also favors the notion that hormonal control was adequate in our patients. Most of the TART were detected in children above 10 years old. It can be suggested that hormones, whose levels is increased in puberty, such s LH, are additional stimulators of tumour growth. The presence of LH receptors in testicular tumour tissue supports this hypothesis (24).

It is known that TART have no malignant features. Therefore, there seems to be no need to treat or remove the tumours at an early stage. However, because of the localization of the tumours in the rete testis, the tumours may compress the seminiferous tubules leading to

obstructive azoospermia and irreversible damage of the testes (2,25). Therefore, it is important to detect and treat the tumours before permanent damage of the testis has occurred. Because the growth promoting factors are still unclear, strategies to prevent tumour growth are difficult to define. It has to be investigated whether testis-sparing surgery at an early stage may prevent irreversible testicular damage in later life.

In summary, our study shows that TART are frequently found already in young CAH children. Based on the absence of signs of gonadal dysfunction in our group of children it seems that gonadal dysfunction, as frequently reported in adult patients, develops after childhood in patients with longstanding tumours. Further studies are necessary to determine whether it is possible to prevent testicular damage by surgical removal of the tumours already in childhood.

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Chapter 6

Ovarian adrenal rest tumours in congenital adrenal hyperplasia – a case report

Chapter 6

Ovarian adrenal rest tissue in congenital adrenal hyperplasia – a case report

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Abstract

We report a case of a young girl who died in an Addisonian crisis due to previously undiagnosed congenital adrenal hyperplasia (CAH), in whom at post mortem histopathological examination ovarian adrenal rest tissue was detected. This is a very rare complication in female CAH patients with only 2 cases reported so far.

Introduction

In male patients with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency, testicular adrenal rest tumors have frequently been described. These tumors are often associated with impaired spermatogenesis and Leydig cell failure (1-7). The prevalence of these tumors in male postpubertal CAH patients is reported as high as 90% (4,5). Analogous to testicular adrenal rest tumors ovarian adrenal rest tumors have been described but only in two single case reports (8,9).

We report a third case of a young girl, who died in an Addisonian crisis due to previously undiagnosed CAH, in which at post mortem examination ovarian adrenal rest tissue was detected.

Case report

While studying the family history of a patient with CAH the following case history from 1962 came to our attention in whom post mortem ovarian tissue was found to contain aberrant adrenal tissue:

The patient, a girl, was admitted to the hospital at the age of 4 months because of failure to thrive and vomiting. Clinical examination revealed clitoromegaly and a pigmented skin, which was most prominent around the nipples. The sodium concentration in serum was 115 mmol/l and the potassium concentration was 8,6 mmol/l.

Because of these findings CAH due to 21 hydroxylase deficiency was suspected. Although the girl was treated immediately with corticosteroids and fluid replacement she died 1 day later.

Histopathology

Revision of the slides of the ovaries revealed multiple nodules varying in diameter, the largest being 0.5 cm. They were situated in the medullary/hilar region of the ovary, partly covered by normal ovarian cortex containing primordial follicles and a few secondary follicles.

The nodules were localized in close approximaty of each other but were still isolated, surrounded by a very delicate fibrous rim of compressed stroma. The nodules showed an

organized structure with centripetal orientation of sinusoids. The constituting cells had a moderate amount of compact granular eosinophilic cytoplasm, sometimes with gross vacuolation. The nuclei were round and monomorphic with small or inconspicuous nucleoli. In zonal arrangement the cells tended to become smaller towards the center of the nodules. Lipofuscin was not apparent.

The cells resembled adrenocortical zona reticularis cells. Although a clear arrangement in zona glomerulosa, zona fasciculata and zona reticularis was not apparent; the nodules had the same histopathology of the hyperplastic adrenal cortex of this child. The adrenals had a broad cortex consisting of comparable eosinophilic cells with sparse lipid vacuolization. A clear zonal demarcation was not visible.

Figure 1. *Overview of ovary (O) and hyperplastic adrenal rest (A) (x12,5).*

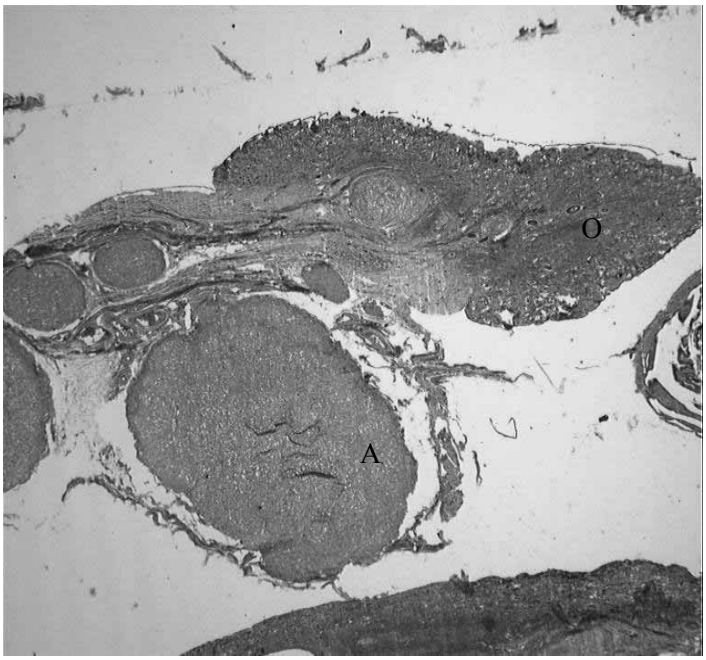


Figure 2. *Hyperplastic nodule showing architectural organization (x50).*

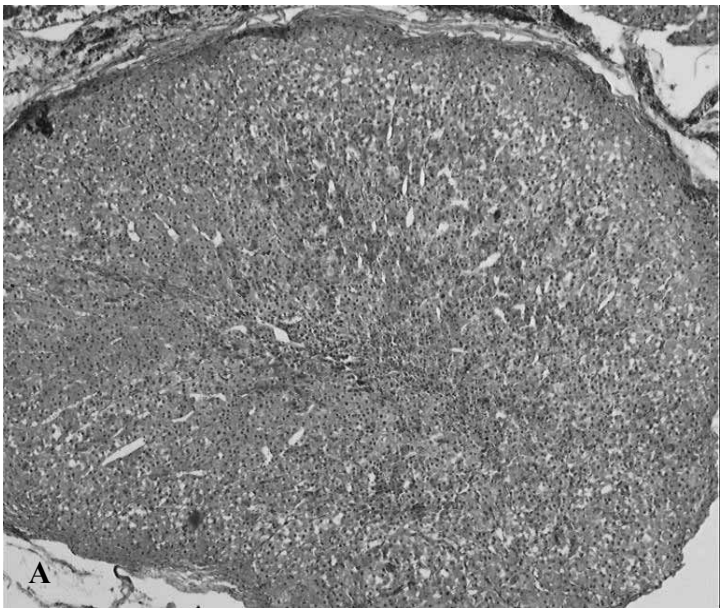


Figure 3. *High magnification of the cells, displaying eosinophilic cytoplasm (x400).*

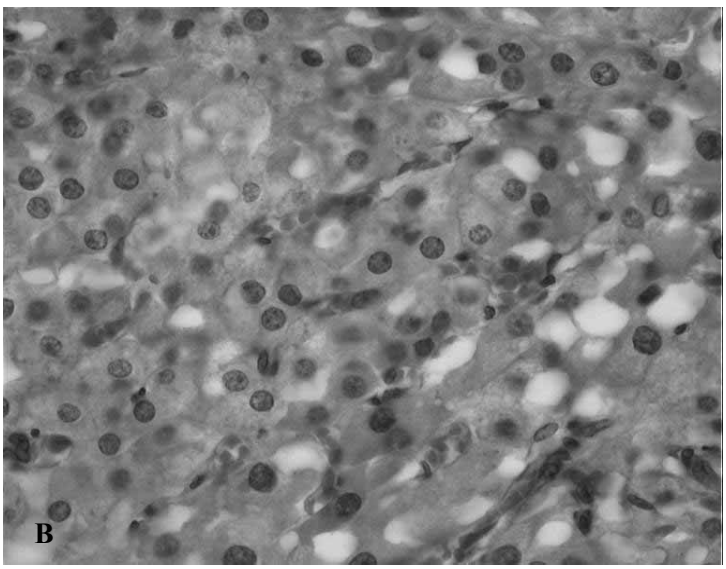
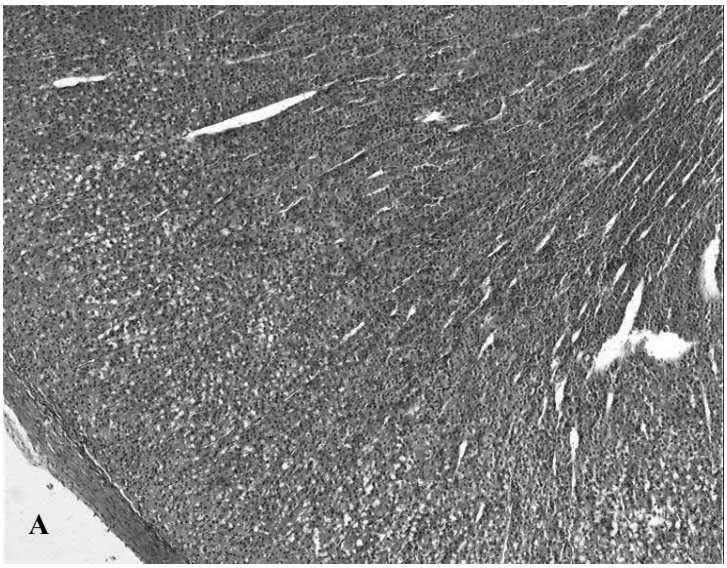
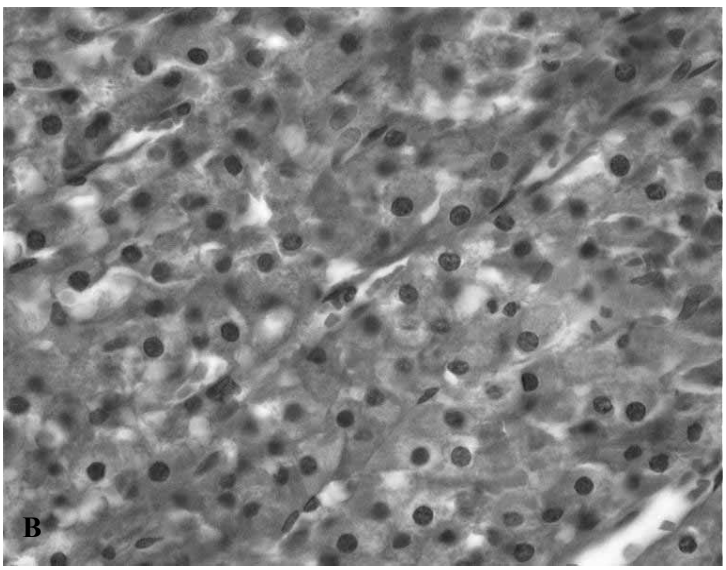


Figure 4. *Hyperplastic adrenal cortex (A, x50), showing at higher magnification (B, x400) the same type of cells.*





Discussion

During the embryological development, the adrenal glands develop in the immediate vicinity of the gonads (10,14). Aberrant adrenal tissue may descend with the testis or ovary along the course of their supplying arteries and end up in the testes, celiac plexus or ovaries. Aberrant adrenal tissue has been identified in 7.5% of normal testes (10). The cells are thought to retain their potential for glucocorticoid production due to ACTH stimulation. Therefore the aberrant adrenal tissue may grow during periods of elevated plasma ACTH levels and may regress when ACTH levels decrease after intensive glucocorticoid treatment as observed in patients with CAH (15-19). Testicular tumors are also described in other patients who have elevated ACTH levels, such as in Nelson's syndrome or Addison's disease (20,21).Testicular tumors found in males with CAH are considered to consist of such adrenal like tissue because of their steroid producing properties and their responsiveness to ACTH as described above. These testicular tumors have been described frequently (1,7). Biochemical studies of testicular tumors in CAH showed adrenal specific 11 ß-hydroxylase activity (22). Steroid measurements in blood drawn from spermatic veins demonstrated cortisol production by the tumors (23,25). Macroscopically the tumors consist of brown-yellow confluent nodules separated by bands of fibrous tissue (26,27). Microscopically the cells form nests separated by bands of dense fibrous tissue. The cells are large and polygonal with fine granular eosinophilic cytoplasm and a round nucleus with a prominent nucleolus. Reinke crystalloids, which can be found in 25-40% of Leydig cell tumors, are not presented (22,26,28). Electron microscopy shows multiple mitochondria and endoplasmatic reticulum beside microvilli and fatdrops, which are typically for steroid producing tissue (3, 29). It seems that ovarian adrenal rest tumors are rare compared with testicular adrenal rest tumors. In the literature only two case reports of histological detected adrenal rest tumors in the ovaries in woman with CAH are reported. Russo et al. described a case of a 15-year-old girl with CAH in whom medical control was poor (8). Because bilaterally enlarged ovaries were found with ultrasound, biopsy of the ovaries was performed.

The histopathological findings were characteristic of adrenocortical tissue. Al-Ahmedin et al. described a case of a 36-year-old woman with CAH who presented with progressive virilising symptoms and very high levels of androstenedione and testosterone (9). Bilateral ovariectomy was performed because ultrasound examination revealed the presence of ovarian masses. The pathological findings were similar to these in testicular adrenal rest tumors: nests of large cells separated by bands of collagen with the characteristic features of steroid producing cells.

After operation the testosterone level normalized. In another case report of Zachmann et al. a woman with CAH was suggested to have adrenal rest tumors in the ovaries because of persistent virilisation after adrenalectomy. However the presence of this tissue was not definitively confirmed (30).

Stikkelbroeck et al. searched for aberrant adrenal tissue in the gonads of male and female CAH patients with ultrasonography and MR (7,31). In none of the 13 female patients ovarian adrenal rest tumors could be detected whereas in 16 of the 17 male patients adrenal rest tumors were found. A possible explanation for this difference in prevalence can be that lesions in testicular tissue are much more easily detectable because the surrounding testicular tissue is very homogeneous whereas ovarian tissue is more heterogeneous on ultrasound (5). The presence of small hypoechoic follicles might prevent detection of adrenal rest tumors by ultrasonography. However, also ovarian MR studies showed no ovarian tumors suggesting that the prevalence of adrenal rests in the ovaries is less high than in the testes (31).

Probably in the female patient other locations than the ovaries are more important such as the broad ligament or the celiac plexus. Possibly other methods than ultrasonography and MR such as scintigraphic investigations may be more helpful in the detection of aberrant adrenal rest tissue.

In our case the cells in the ovarian nodules as found by post mortem examination, were characteristic of adrenocortical zona reticularis cells. The same histopathology was found in the hyperplastic adrenal cortex of this child, suggesting that the ovarian nodules are of adrenal origin. The microscopical aspect of the cells with all characteristic features of steroid producing cells was similar to the histological findings of the ovarian adrenal rests described in the literature (8,9).

In conclusion, ovarian adrenal rest tumors are rare with only two cases described so far. Here we report a third case of a child with CAH and ovarian adrenal rests. It remains unexplained why the incidence of these tumors in female CAH patients is so low compared with those of adrenal testicular tumors in men.

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Chapter 7

Summary and general discussion

7.1. Summary

In male patients with congenital adrenal hyperplasia (CAH) testicular adrenal rest tumours (TART) are frequently present. The tumours can lead to obstruction of the seminiferous tubules and gonadal dysfunction. Therefore, knowledge about the etiology, the pathogenesis and the functional features of these tumours is very important to develop strategies for tailor-made treatment of these tumours. In this thesis we studied several aspects of TART in CAH patients focusing on the etiology, the clinical consequences and treatment options.

Chapter 1.1 gives a general overview of CAH. CAH is one of the most common inherited disorders affecting adrenal steroid synthesis due to an enzyme deficiency. In more than 90% of the cases CAH is caused by CYP21 (21-hydroxylase) deficiency. Other enzyme deficiencies are not subject of this thesis.

CYP21 deficiency results in an impaired production of cortisol and mostly also of aldosterone. Consequently, pituitary ACTH production is increased leading to hyperplasia of the adrenal glands and overproduction of adrenal androgens with prenatal virilisation of the female external genitalia. The impaired production of cortisol and aldosterone may lead to an Addisonian crisis with salt wasting and dehydration in early life. In particular male CAH neonates are at risk for the development of severe salt wasting in the second week of life because they do not show abnormalities of the external genitalia at birth and the diagnosis will only become apparent when salt wasting occurs. Therefore, in the last years neonatal screening for CAH has been introduced in many Western countries to prevent life threatening events in affected children.

CAH is caused by mutations in the CYP21A2 gene located on chromosome 6p21.3, close to a 98% homologous pseudogene CYP21A2P. This pseudogene contains several inactivating mutations that may be transferred to the active CYP21 gene by gene-transfer (about 60% of the mutations). Approximately another 30% of the mutations found in CAH patients are caused by unequal cross-over during meiosis that completely deletes the gene. 1-2% of the mutations are spontaneous mutations not carried by one of the parents.

The phenotype depends on the degree of enzyme deficiency with an overall genotype-phenotype correlation of 80%. In the most severe type, there is a complete loss-of-function of the CYP21 enzyme resulting in severe salt wasting and virilisation of the external genitalia in the affected girls (classical salt wasting type of CAH). Several point mutations result in an

enzyme activity of approximately 1% that allows sufficient aldosterone synthesis to prevent salt wasting but with impaired cortisol synthesis and increased adrenal androgen synthesis with prenatal virilisation of the girls (simple virilising type of CAH). Other mutations result in a 20-50% residual enzyme activity. These patients produce sufficient amounts of cortisol and aldosterone but still have increased adrenal androgen secretion leading to a clinical picture of pseudo puberty in childhood or acne, hirsutism and menstrual disorders in adulthood (late onset type of CAH).
The incidence of the classic type of CYP21 deficiency in the Netherlands is 1:12 000. The incidence of the late onset type of CAH is much higher (1: 1700). The diagnosis CAH due to CYP21 deficiency can be made by measuring the steroid precursors 17-hydroxyprogesterone and androstenedione or its metabolites in serum, saliva or urine and can be confirmed by mutation analysis.

Treatment of CAH consists of glucocorticoid substitution, thereby also suppressing pituitary ACTH release and subsequently adrenal androgen production. Patients with additional aldosterone deficiency require also supplemental mineralocorticoids. Affected girls mostly need surgical correction of the external genitalia in the first year of life. In pregnancies at risk for a girl with the classic type of CAH and virilisation in utero, suppression of adrenal androgens in utero can be achieved by administering high doses of dexamethasone to the mother thereby preventing or reducing prenatal virilisation of the affected child.
In adult CAH patients the most important complication is infertility. In both CAH males and females fertility rate is reduced. **Chapter 1.2** gives an overview of the causes and pathophysiology of gonadal dysfunction and infertility in CAH patients.

The most important cause of infertility in male patients is the presence of TART. The reported incidence of TART in adult male CAH patients varies between 0 and 94% dependent on patient selection and method of detection. The tumours are always located in the rete testis and may lead to long-standing obstruction of the seminiferous tubules with testicular damage and infertility. Poor hormonal control may be an important factor in tumour growth. Therefore, in some cases optimizing glucocorticoid treatment is reported to lead to a decrease in tumour size and improvement of gonadal function. Other studies, however, do not show any correlation between hormonal control and tumour growth. Recently, two studies described testis-sparing surgery in glucocorticoid unresponsive testicular tumours. Detailed information about the effect of surgical treatment on gonadal function was not reported. A

second cause of gonadal dysfunction in poorly controlled male CAH patients is the development of hypogonadotropic hypogonadism due to high serum levels of adrenal androgens, which are partly aromatized to oestron. Both androstenedione and oestron can suppress the hypothalamic-pituitary-gonadal axis leading to hypogonadotropic hypogonadism. Optimizing glucocorticoid therapy leads to normalization of adrenal androgens in blood and subsequently of gonadotropin concentrations and improvement of gonadal function.

In female CAH patients several factors have been suggested to contribute to impaired fertility. Adrenal androgen overproduction can directly or indirectly affect gonadal function. Androgen excess may inhibit ovarian folliculogenesis, endometrial proliferation and may lead to failure of endometrial breakdown, which results in menstrual disorders. Additionally, elevated progesterone secretion by the adrenals may cause involution of the endometrium and impermeability of the cervical mucus. Similar to in male patients, increased androgen levels can result in hypogonadotropic hypogonadism. Besides the endocrine factors the mechanical effects of virilisation and genital surgery in early life may also play a role in impaired fertility.

In **chapter 2.1** the functional features of TART in male CAH patients are described. TART are thought to arise from aberrant adrenal cells descended in the embryological period together with the testes. However, until now the etiology and functional features of these tumours are not completely understood. In the past only a limited number of studies, mainly in single patients, were performed to investigate functional aspects of TART. The clinical observation that high doses of glucocorticoids can reduce tumour size most probably due to suppression of ACTH secretion and the presence of adrenal specific steroids in spermatic venous blood in patients with TART suggests that the tumours consist of adrenal-like cells. Therefore, we studied the steroid producing features of TART in more detail in eight adult male patients with longstanding bilateral TART who were treated with testis-sparing surgery.

All patients were infertile and most of them had been treated with high dosages of glucocorticoids in the past in an attempt to reduce tumour size. In all but one patient spermatic veins were canulated during surgery and blood samples were collected to measure the adrenal specific steroid 21-deoxycortisol (21DF) and the steroids 17-hydroxyprogesterone (17OHP) and androstenedione (A). The same parameters were measured in simultaneously taken peripheral blood. Furthermore, the removed tumour tissue was investigated microscopically and mRNA concentrations of the adrenal specific enzymes CYP11B1 and

CYP11B2 and of the receptors for ACTH and angiotensin II (AII) were measured by RT-PCR. In all but one patient, in whom canulation of the spermatic veins was not completely successful, the concentration of the adrenal specific steroid 21DF in the spermatic veins was significantly higher than the concentration in the simultaneously taken peripheral blood samples. Additionally, at the mRNA level, the adrenal specific enzymes CYP11B1 and CYP11B2, and ACTH and AII receptors were present in all testicular tumours, strongly suggesting that the tumours consist of adrenal-like tissue. We hypothesize that in line with the presence of AII receptors in all tumours, AII is an additional factor responsible for tumour growth in CAH patients with poor hormonal control.
All tumours were located within the rete testis leading to mechanical obstruction of the seminiferous tubules and probably to testicular damage. So far, no detailed data about the quality of the residual testicular parenchyma in CAH patients with TART has been reported. Therefore, in seven of the eight operated patients, testicular biopsies were taken for histological evaluation. The results are described in **chapter 2.2.** All testicular biopsies showed a decrease in tubular diameter with peritubular fibrosis and in four patients tubular hyalinization suggesting irreversible damage of testicular parenchyma. The germative layer showed decreased spermatogenesis and reduced Johnson scores explaining persistent infertility in our patients.

Different treatment strategies to reduce testicular tumour size and to prevent gonadal dysfunction in male CAH patients have been described. Because of the presence of ACTH receptors in tumour cells it is suggested that treatment with high doses of glucocorticoids may lead to suppression of ACTH secretion and possibly reduction of tumour size. In **chapter 3.1** we describe a male CAH patient with bilateral TART and azoospermia who was treated with short periods of dexamethasone in an attempt to reduce tumour size and improve testicular function. During dexamethasone treatment he showed decrease in tumour size and improvement of sperm quality and spontaneous conception was achieved.

High doses of glucocorticoids, however, may have severe side effects and several studies show that optimizing glucocorticoid therapy does not always reduce tumour size or restore testicular function. Therefore, testis-sparing surgery has been proposed for the treatment of TART in CAH patients but the effect on gonadal function is not known. In our group of 8 patients tumour volume, symptoms and pituitary-gonadal function was evaluated before and after surgery. The results are described in **chapter 3.2.** In none of the patients residual

tumours were found on MRI after surgery. Two patients reported pain and discomfort that disappeared after testis-sparing surgery. Parameters of pituitary-gonadal function did not improve after surgery. Semen analysis showed azoospermia in 5 patients and oligospermia in one patient without improvement following surgery. All patients had persistently low inhibin B concentrations. We concluded that in patients with longstanding TART testis-sparing surgery did not improve pituitary-gonadal function and fertility.

In all operated patients testicular MRI was performed before, and 6 and 22 months after surgery. In **chapter 4** the radiological evaluation of TART and of the residual testicular tissue is described. No residual tumours were seen on postoperative MRI. In all patients residual testicular parenchyma had significantly decreased in volume 22 months after the surgery suggesting irreversible damage of testicular parenchyma.

The prevalence of TART in male CAH children and the consequences for gonadal function in childhood are not completely known. Therefore, the incidence of TART was investigated by scrotal ultrasound in 34 male CAH children between 2 and 18 years old who were followed regularly in our outpatient clinic. In children with TART LH, FSH, inhibin B and testosterone concentrations were measured in serum and compared with the levels of these hormones in children without TART in order to evaluate gonadal function. The results are described in **chapter 5**. In our study group TART was found with an incidence of 24%. Two children with TART were between 5 and 10 years old and six children were above 10 years old. In two of the eight children bilateral tumours were found. In the children with TART LH, FSH, testosterone and inhibin B concentrations were in the same range as in the children without TART. We concluded that TART can already be present in childhood and that in our group of children with TART gonadal function was not yet disturbed.
In contrast to the findings in male CAH patients ovarian adrenal rest tumours in female CAH patients are rare. In the literature only two case reports of histologically detected ovarian adrenal rests in female CAH patients are reported. In earlier studies we searched for ovarian adrenal rest tissue in thirteen female CAH patients with ultrasound and MRI. In none of these patients ovarian adrenal rest could be detected. In **chapter 6** a case of ovarian adrenal rest tissue is described in a young girl who died in an Addisonian crisis due to previously undiagnosed CAH in whom at post mortem histopathological examination ovarian adrenal rest tissue was detected.

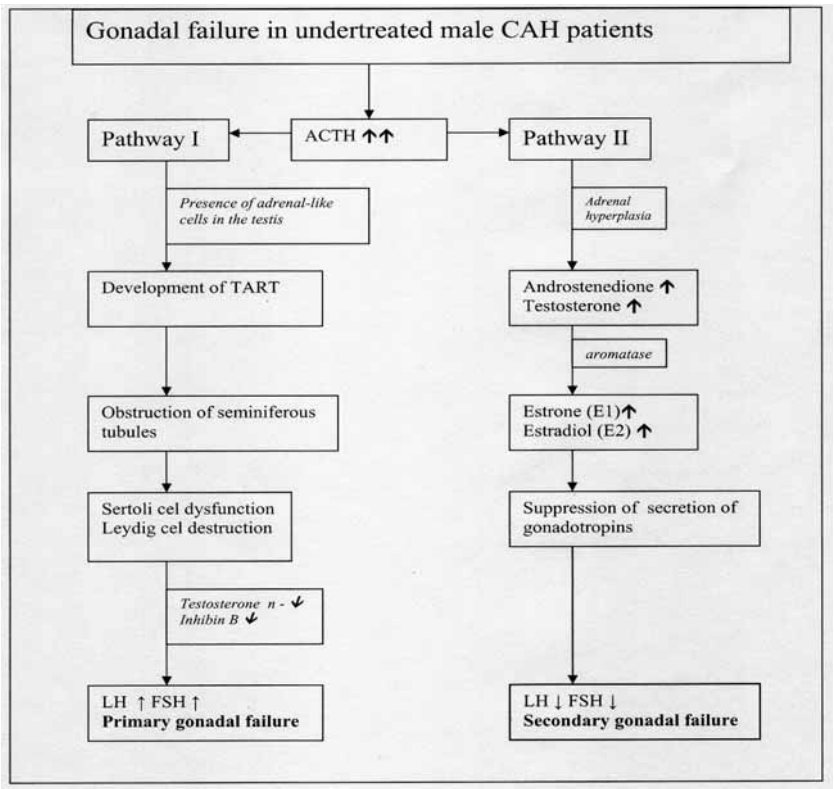
7.2. General discussion

Gonadal dysfunction in male CAH patients

Infertility is a serious complication in male CAH patients. As described in chapter 1.2 two mechanisms leading to gonadal dysfunction can be identified. Both mechanisms are induced by elevated ACTH levels (fig. 1).

1. Primary gonadal failure (hypergonadotropic hypogonadism) due to testicular adrenal rest tumours (TART).
2. Secondary gonadal failure (hypogonadotropic hypogonadism) due to suppression of LH and FSH secretion by elevated serum levels of androstenedione and testosterone, which are partly aromatized to estrone and estradiol.

Figure 1. *Mechanisms of gonadal dysfunction in male patients with congenital adrenal hyperplasia and poor hormonal control. Mechanism I: primary gonadal failure caused by obstruction of the seminiferous tubules due to TART. Mechanism II: secondary gonadal failure due to suppression of the pituitary-gonadal axis.*



It should be realized that both pathophysiological mechanisms can act together and that it can be difficult to distinguish which mechanism(s) is/are operational because the elevated LH and FSH concentrations in primary gonadal failure may be suppressed by elevated estrone and androstenedione concentrations. Therefore, LH and FSH levels should be interpreted with caution in CAH patients. The study described in chapter 3.2 suggests that in male CAH patients inhibin B is a useful marker for the evaluation of Sertoli cell function. This parameter may be used as well in the evaluation of gonadal function in prepubertal children (chapter 5).

Hypogonadotropic hypogonadism due to suppression of gonadotropins in poorly controlled patients can be effectively treated with increasing the dose of glucocorticoids to suppress ACTH and consequently androstenedione levels. However, frequently patients are reluctant to take high doses of glucocorticoids because of side effects. Especially in these cases a new treatment option could be treatment with aromatase inhibitors. This class of drugs inhibits the conversion of androstenedione and testosterone to estrone and estradiol. The decrease in estrogen levels may lead to normalization of the suppressed gonadotropins and improvement of gonadal function. Further studies should be performed to support this hypothesis.

The origin and pathogenesis of TART

Our studies reveal that TART contain adrenal specific enzymes and produce adrenal specific hormones suggesting that these tumours arise from adrenal-like cells. The presence at the mRNA level of ACTH and angiotensin II (AII) receptors in the adrenal rest tumours as described in chapter 2.1 supports this hypothesis. The adrenal glands develop in the immediate vicinity of the gonads and cells originally destined to become adrenocortical cells may nestle within the rete testis (1,2). In the literature a prevalence of up to 15% of such so called adrenal rest cells in the testes of healthy neonates is reported (3,4). However, this prevalence is probably underestimated because single adrenal-like cells or small cell groups are very difficult to detect.

It is thought that poor hormonal control, leading to high blood levels of ACTH (and/or AII), is an important factor in the pathogenesis of TART inducing hypertrophy and hyperplasia of adrenal-like cells within the testis (5,6). However, TART are also found in adequately treated patients, whereas some poorly controlled male CAH patients do not develop TART despite chronically elevated ACTH levels (7,8). The most plausible explanation for this observation is

that in the embryological period not in all males aberrant adrenal cells nestle in the testes. The presence of these aberrant adrenal cells within the testis is the most probable prerequisite for the development of TART explaining the often observed discrepancy between the development of TART and hormonal control. It is likely that CAH patients without adrenal rest cells within the testis will never develop TART.

Our observation, that TART can already be detected in early childhood even in adequately treated patients, suggests that when adrenal rest cells are present within the testis, even mildly or intermittently increased ACTH (and AII) concentrations may induce proliferation of these cells within the testis. Poor hormonal control with high ACTH levels may accelerate this process. So, both the concentrations of and the duration of exposure to growth promoting factors are probably important in the pathogenesis of tumour growth. Furthermore, it can be hypothesized that the pubertal rise of LH may give an additional stimulation of tumour growth as LH receptors are found in TART (9), which may explain the increased prevalence of TART in pubertal and postpubertal CAH patients. Detailed studies focusing on the effect of ACTH, AII and LH in young, male CAH patients are needed to determine the role of these factors in the development of TART.

The natural history of adrenal rest cells in healthy neonates is insufficiently known, but it is suggested that spontaneous regression occurs within the first years of life (7,8). It can be hypothesized, that spontaneous regression of these adrenal rest cells may also occur in male CAH children when growth promoting factors such as ACTH (and AII) are effectively suppressed in the first years of life. However, this treatment strategy conflicts with the negative effect of high doses of glucocorticoids on growth velocity in the first years of life. Therefore, the optimal medical treatment strategy in the first years of life is still not clear.

Because only patients in whom aberrant adrenal cells have been nestled in the testes in the embryological period are supposed to be at risk for developing TART, it would be very important to identify these patients as early as possible. Nowadays, adrenal rest cells can only be detected after substantial growth.
In the future, new sensitive imaging techniques may help to detect these adrenal rests in the first years of life. If this is possible, patients with adrenal rest cells within the rete testis could be monitored and treated more intensively, whereas in patients without adrenal rests unnecessary ultrasound follow up and aggressive treatment strategies could be avoided.

Classification of TART

In our study group of 8 adult male CAH patients, all TART were located within the rete testis. The same observation was made in our group of 8 asymptomatic CAH children with TART detected by ultrasound. In this group of children we did not find signs of gonadal dysfunction. However, in the adult CAH patients, studied in chapter 2.2, TART had led to irreversible gonadal dysfunction. Histologically, these longstanding tumours were characterized by sheets of confluent cords of large polygonal cells with abundant eosinophilic cytoplasm, separated by dense fibrous tissue strands. The rete testis was always found to be compressed. Based on these observations, we propose that the development and growth of TART can be divided in five different stages based on histological criteria of TART and the surrounding testicular parenchyma (table 1, fig. 2):

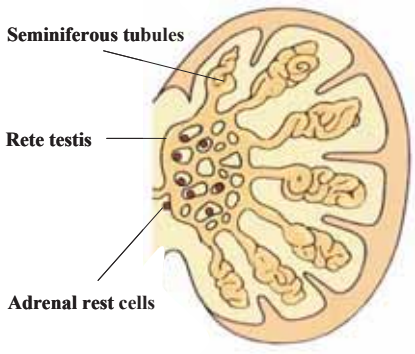
Table 1. *Proposed classification of TART*

	Histological description	**Reversibility**	**Treatment options**
Stage 1	Presence of adrenal rests within the rete testis	+++	-
Stage 2	Hypertrophy and hyperplasia of adrenal rest cells due to growth stimulating factors (for example ACTH, AII)	+++	Optimizing glucocorticoids Surgery?
Stage 3	Further growth of the adrenal rest cells with (reversible) compression of the rete testis	++	Optimizing glucocorticoids Surgery?
Stage 4	Induction of fibrosis and focal lymphocytic infiltrates	-/+	Surgery?
Stage 5	Irreversible damage of testicular parenchyma. Parts of the tumour are replaced by adipose tissue	-	-

Figure 2. *Schematic view of proposed classification of testicular adrenal rest tumours*

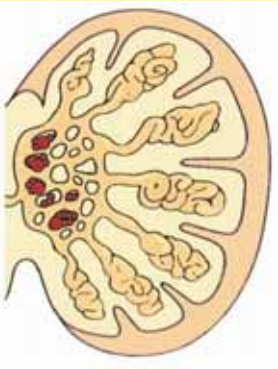
Stage 1

Adrenal rest cells present within the rete testis



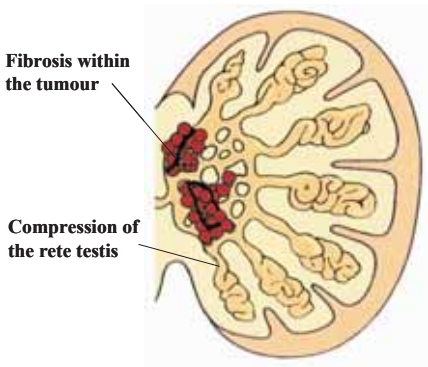
Stage 2

Hyperplasia and hypertrophy of adrenal rest cells



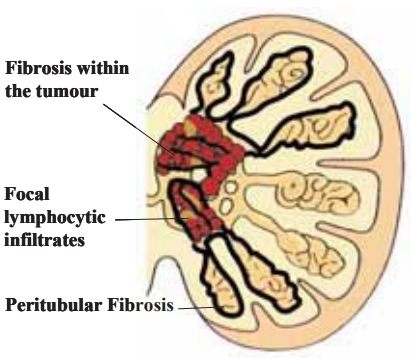
Stage 3

Further growth of the adrenal rest cells with compression of the rete testis



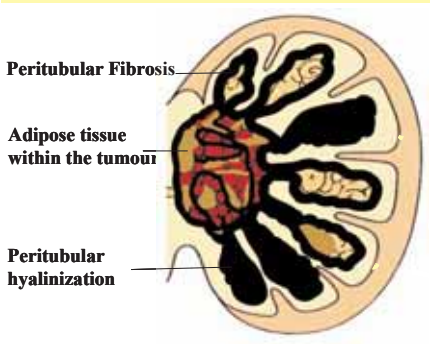
Stage 4

Induction of fibrosis and focal lymphocytic infiltrates



Stage 5

Irreversible damage of testicular parenchyma



Stage 1: This stage can be defined as the presence of adrenal rest cells within the rete testis, not detectable by scrotal ultrasound. In healthy boys these cells probably regress in the first years of life.

Stage 2: In CAH patients, the adrenal rest cells may proliferate in the presence of increased concentrations of growth promoting factors such as ACTH (and possibly also of AII). In this stage the adrenal rest cells may become visible by ultrasound as hypoechogenic lesions. The age of onset of cell growth may depend on the cumulative exposure to ACTH (and AII) concentrations over time and the number of ACTH (and AII) receptors on the adrenal rest cells.

Stage 3: Further growth of the adrenal rest cells will compress the rete testis. In pubertal or postpubertal CAH patients, oligo- or azoospermia may already be found due to obstruction of the seminiferous tubules. Signs of gonadal dysfunction such as decreased inhibin B and increased FSH and LH levels may also be present. As demonstrated in chapter 3.1 tumour size may still be reduced by high dosages of glucocorticoids. However, it is expected that tumour growth will continue after decreasing the dose of glucocorticoids.

Stage 4: Further hypertrophy and hyperplasia of the adrenal rest cells with progressive obstruction of the rete testis may lead to induction of fibrosis within the tumour and focal lymphocytic infiltration. Several small tumours within the rete testis will confluate, forming a single lobulated structure separated from the residual testicular tissue by fibrous strands. In this stage high doses of glucocorticoids are probably no longer effective in decreasing tumour size because parts of the tumours consist of fibrous tissue and/or because the adrenal rest cells may dedifferentiate in time with loss of ACTH dependency.

Stage 5: Chronic obstruction subsequently will lead to destruction of the surrounding testicular parenchyma with irreversible damage of the testis.
Further studies are necessary to validate the proposed classification of TART.

Treatment of TART

The proposed classification of TART may help in the decision which treatment option is useful in individual patients. Scrotal ultrasound and biochemical analysis may help to

determine the stage of TART in individual CAH patients: in TART stage 2 hypoechogenic round lesions may become visible within the rete testis. An echogenic focus within the lesion may indicate fibrotic strengs in the tumours as present in TART stages 3 to 5. From stage 3 elevated gonadotropins and low inhibin B concentrations may be found indicating compression of the seminiferous tubules. TART stages 2 and 3 may be successfully treated by increasing the dose of glucocorticoids. However, it may be that this treatment leads only to temporary improvement of the obstruction because tumour growth may start again after lowering the glucocorticoid dose (chapter 3.1). Furthermore, high doses of glucocorticoids may have severe side effects and therefore, the majority of the patients will not accept this treatment option. However, optimizing glucocorticoid medication especially in patients with poor hormonal control is important to determine whether tumour growth is reversible (stage 3). In stage 4 increasing the dose of glucocorticoids is probably no longer effective in decreasing tumour size, but removal of the tumour may prevent further testicular damage. In stage 5 no treatment options are useful to improve gonadal function because of irreversible gonadal damage. Therefore, before surgery is considered, testicular biopsies are advised to evaluate the quality of the surrounding testicular parenchyma.

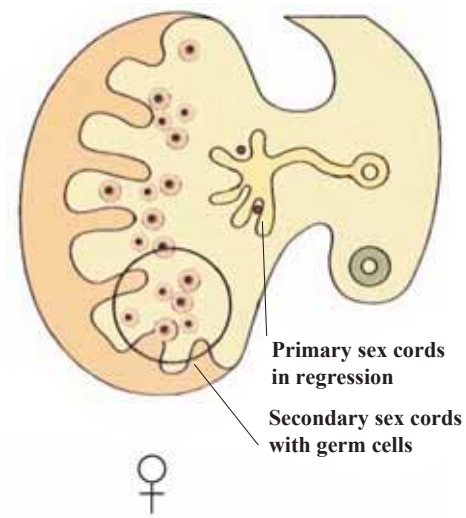
In our study, eight adult CAH patients with TART were treated with testis-sparing surgery (chapter 3.2). Postoperatively it appeared that in all eight patients irreversible damage of the testicular parenchyma was present (chapter 5). From our experience we now conclude that in this stage the only indication for surgery is the relief of pain and discomfort caused by TART. Further studies already in childhood age are needed to investigate whether surgery in stage 2, 3 and 4 may prevent irreversible damage of the testes. Hopefully, in order to prevent destruction of residual testicular parenchyma introduction of new surgical techniques may facilitate the surgical treatment of the tumours already in childhood. As long as medical and surgical treatments of TART are far from perfect, patients should be informed about the negative effects of TART on fertility and cryopreservation of semen should be offered as soon as possible.

Adrenal rest tumours in female CAH patients

In our study we reported a case of ovarian rests in a young girl with CAH. Interestingly, in contrast to TART in male CAH patients, ovarian adrenal rest tumours in female CAH patients seem to be very rare. The reason for this low prevalence of ovarian adrenal rests is not known.

We propose the following hypothesis to explain the striking difference in the prevalence of gonadal adrenal rest tumours between males and females: in the 5th week of the embryological period the gonadal ridge has the potential to develop to either a male or female gonad depending on the karyotype of the migrating primordial germ cells. In both male and female embryos the mesonephric duct forms primary sex cords. In the male embryo these primary sex cords penetrate into the medulla of the developing testis becoming the rete testis and seminiferous tubules. In this primary sex cords aberrant adrenal cells from the nearby developing adrenal cortex may easily nestle. In contrast, in the female embryo, the primary sex cords regress and secondary (cortical) sex cords will develop together with thickening of the surface epithelium (fig.3). Therefore, in females aberrant adrenal cells nestled in the primary sex cords will also regress.

Figure 3. *Development of the female fetal gonads. Note the proposed presence of adrenal rest cells (red points) within the primary sex cords. It can be hypothesized that in the female gonad the adrenal rest cells will regress together with the primary sex cord.*



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Chapter 8

Samenvatting

Zusammenfassung

8.1. Samenvatting

Het ontstaan van bijnierresttumoren in de testes is een vaak voorkomende complicatie bij mannen met het adrenogenitaal syndroom (AGS). Deze tumoren kunnen leiden tot chronische obstructie van de tubuli seminiferi en gonadale dysfunctie. Een goede kennis van de pathogenese en de functionele eigenschappen van deze tumoren is essentieel om optimale behandelingsstrategieën te ontwikkelen. In dit proefschrift worden verschillende functionele eigenschappen en klinische aspecten van bijnierresttumoren beschreven. De Engelse benaming van deze tumoren is **T**esticular **A**drenal **R**est **T**umors en daarom worden in dit proefschrift deze tumoren kortweg TART genoemd.

Hoofdstuk 1.1. geeft een overzicht van het ziektebeeld AGS. AGS is een van de meest voorkomende autosomaal-recessief overervende ziekten en wordt veroorzaakt door deficiëntie van één van de enzymen die betrokken zijn bij de synthese van cortisol en aldosteron in de bijnier. In meer dan 90% van de gevallen wordt AGS veroorzaakt door een deficiëntie van het CYP21 (21-hydroxylase) enzym. Hierdoor is er een stoornis in de synthese van cortisol en meestal ook van aldosteron in de bijnierschors met als gevolg een onvoldoende negatieve terugkoppeling op de ACTH secretie door de hypofyse. Overmatige ACTH secretie stimuleert de bijnier en leidt tot bijnierhyperplasie (vandaar de Engelse benaming "congenital adrenal hyperplasia"). De androgeensynthese is niet gestoord door de enzymdeficiëntie en door de hoge ACTH concentratie is de productie van androgenen in de bijnier sterk verhoogd. Hierdoor treedt er al prenataal virilisatie op van de genitalia externa bij aangedane meisjes.
De gestoorde cortisol- en aldosteronproductie leidt postnataal tot een Addison crisis met zoutverlies en dehydratie. Vooral jongens hebben een verhoogd risico op het ontwikkelen van een Addison crisis 5 – 10 dagen na de geboorte omdat zij bij de geboorte geen symptomen vertonen (d.w.z. geen afwijkingen van de uitwendige genitalia) en dus niet tijdig gediagnosticeerd kunnen worden. Om levensbedreigende situaties te voorkomen is daarom in veel Westerse landen neonatale screening op AGS geïntroduceerd.

AGS wordt veroorzaakt door mutaties in het CYP21A2 gen dat codeert voor het enzym CYP21. Het gen is gelegen op chromosoom 6p21.3. Het gen is voor 98% identiek aan een inactief pseudogen (CYP21p) dat in de directe omgeving van het actieve CYP21A2 gen ligt. In het CYP21p gen bevinden zich een aantal mutaties, die het gen volledig inactiveren. De mutaties, die bij AGS patiënten gevonden worden, zijn in meer dan 60% van de gevallen via

gen-conversie overgebracht van het niet functionele CYP21p gen naar het functionele CYP21A2 gen. Het precieze mechanisme van deze gen-conversie is niet bekend. In meer dan 30% van de gevallen leidt een ongelijke cross-over tijdens de meiose tot een complete deletie van het CYP21A2 gen. Spontane mutaties worden in 1 - 2% van de gemuteerde allelen gevonden.

Het klinisch beeld is afhankelijk van de ernst van de enzymdeficiëntie met een genotype-fenotype correlatie van ongeveer 80%. Een complete deficiëntie van het CYP21 enzym leidt tot ernstig zoutverlies na de geboorte en virilisatie van de vrouwelijke genitalia externa (klassieke zoutverliezende vorm van AGS). Enkele puntmutaties leiden tot een rest enzymactiviteit van 1 - 2% met nog voldoende aldosteronsynthese ter voorkoming van zoutverlies maar met cortisoldeficiëntie en verhoogde productie van adrenale androgenen en virilisatie van de aangedane meisjes (simple virilising vorm van AGS). Andere mutaties leiden tot een rest enzymactiviteit van 30 - 50%. Deze patiënten produceren voldoende cortisol en aldosteron maar hebben verhoogde concentraties van adrenale androgenen, die op de kinderleeftijd kunnen leiden tot het klinisch beeld van pseudo pubertas praecox en op volwassen leeftijd kunnen leiden tot acne, hirsutisme en menstruatieproblemen (late onset vorm van AGS).

De incidentie van de klassieke vorm van AGS in Nederland is 1: 12 000. De incidentie van de late onset vorm van AGS is hoger en is ongeveer 1: 1700. De diagnose AGS op basis van een CYP21 deficiëntie kan gesteld worden door het vaststellen van verhoogde concentraties van de steroïdprecursors 17-hydroxyprogesteron (17OHP) en androsteendion (A) in serum- of speekselmonsters of van metabolieten van deze steroïden in 24-uurs urine (“steroidprofiel”). De diagnose kan bevestigd worden middels mutatieanalyse. De behandeling van AGS bestaat uit toediening van glucocorticoïden met als doel enerzijds substitutie van cortisol en anderzijds suppressie van ACTH en daardoor suppressie van de androgeenproductie. Bij een aldosterondeficiëntie moeten ook mineralocorticoïden en in het eerste levensjaar vaak ook zout gesuppleerd worden. Bij meisjes met de klassieke vorm van AGS is meestal een operatieve correctie van de genitalia externa noodzakelijk.

Ouders, die beiden drager zijn van een mutatie welke geassocieerd is met de klassieke vorm van AGS, hebben een kans van 12,5% op het krijgen van een geviriliseerd meisje met AGS. In dit geval kan virilisatie van het meisje verminderd worden door de moeder tijdens de

zwangerschap te behandelen met dexamethason (DXM) waardoor de androgeenproductie door de embryonale bijnier geremd wordt.

Infertiliteit is de meest belangrijke complicatie bij volwassen patiënten met AGS. In **hoofdstuk 1.2.** wordt een overzicht gegeven van de oorzaken van gonadale dysfunctie en infertiliteit bij mannen en vrouwen met AGS.
TART is de meest voorkomende oorzaak van infertiliteit bij mannen met AGS. De incidentie van TART ligt tussen 0 en 94%, afhankelijk van de patiëntselectie en methode van detectie. Deze goedaardige tumoren zijn altijd gelegen in de rete testis en kunnen uiteindelijk leiden tot obstructie van de tubuli seminiferi en irreversibele beschadiging van het testisparenchym (hypergonadotroop hypogonadisme). Een tweede oorzaak voor infertiliteit bij mannen is een hypogonadotroop hypogonadisme ten gevolge van suppressie van de hypothalamus-hypofyse-gonaden as door overproductie van bijnierandrogenen.
Bij vrouwen met AGS kunnen een aantal factoren bijdragen aan een verminderde fertiliteit. Verhoogde concentraties van bijnierandrogenen oefenen een negatieve invloed uit op de gonadale functie. De verhoogde concentraties van androgenen kunnen de ovariële folliculogenese en endometriumproliferatie remmen. Verder kunnen verhoogde concentraties van door de bijnier geproduceerd progesteron leiden tot involutie van het endometrium en impermeabiliteit van de cervicale mucus. Evenals bij mannen met AGS kan een verhoogde productie van bijnierandrogenen leiden tot een hypogonadotroop hypogonadisme.
Behoudens endocriene factoren kunnen ook mechanische effecten ten gevolge van virilisatie en chirurgie een rol spelen bij de verminderde fertiliteit bij vrouwen met AGS.

In dit proefschrift worden een aantal studies beschreven bij 8 volwassen mannen met AGS, bilaterale TART en gonadale dysfunctie, die allen geopereerd werden door middel van een testissparende operatie. Beschreven worden de functionele eigenschappen van de tumoren (hoofdstuk 2.1.) het histologische aspect van het omgevende testisweefsel (hoofdstuk 2.2.), de operatieresultaten (hoofdstuk 3.2.) en radiologische kenmerken van de tumoren en het omgevende testesweefsel (hoofdstuk 4). Verder wordt de medicamenteuze behandeling van TART aan de hand van een casus beschreven (hoofdstuk 3.1.) en wordt een studie naar de incidentie van TART op kinderleeftijd beschreven (hoofdstuk 5). Als laatste wordt een meisje met AGS beschreven met een zeldzame ovariële bijnierresttumor (hoofdstuk 6) .

In **hoofdstuk 2.1.** worden enkele functionele aspecten van TART beschreven. De cellen waaruit TART zijn opgebouwd zijn waarschijnlijk afkomstig uit cellen die zich tijdens de embryogenese ontwikkelen tot bijniercellen. Hiervoor pleit de aanwezigheid van bijnierspecifieke hormonen gemeten in de vena spermatica van patiënten met TART en de klinische observatie dat door hoge doseringen glucocorticoïden de tumorgrootte beïnvloed kan worden. De gedachte is dat tijdens de embryonale periode bijniercellen, die zich bevinden in de zich vlak bij de testis ontwikkelende bijnier innestelen in de rete testis en samen met de testis descenderen naar het scrotum. Onder invloed van groeibevorderende factoren zoals ACTH, kunnen deze cellen groeien en hormonaal actief worden. Onderbehandeling van patiënten met hoge ACTH concentraties speelt waarschijnlijk een belangrijke rol bij de groei van TART. Optimalisering van de glucocorticoïdbehandeling leidt bij sommige patiënten tot een afname van de tumorgrootte en verbetering van de testiculaire functie. Andere studies beschrijven echter geen afname van de tumorgrootte na intensiveren van de glucocorticoïdmedicatie.

De etiologie en functionele kenmerken van deze tumoren zijn voornamelijk in case reports gerapporteerd. In de studie beschreven in hoofdstuk 2.1. werden de steroïdproducerende eigenschappen van TART bestudeerd bij acht mannen met AGS en bilaterale testiculaire tumoren, die allen behandeld werden door middel van een testissparende operatie. Alle patiënten waren infertiel en 6 van de 8 patienten waren in het verleden zonder succes behandeld met hoge doseringen glucocorticoïden om de tumorgrootte te verminderen en de gonadale functie te verbeteren. Bij zeven van de acht patiënten werd tijdens de operatie de venae spermaticae gecanuleerd en werd bloed afgenomen ter bepaling van het bijnierspecifieke hormoon 21-deoxycortisol (21DF) en van 17OHP en A. Dezelfde parameters werden bepaald in gelijktijdig afgenomen perifere bloedmonsters. Het verwijderde tumorweefsel werd microscopisch onderzocht. mRNA concentraties van de bijnierspecifieke enzymen CYP11B1 en CYP11B2 en van de ACTH en angiotensine II (AII) receptoren werden gemeten middels polymerase chain reaction (PCR) technieken. In alle onderzochte patiënten met uitzondering van een sampling bij een patiënt bij wie canulatie van de sterk atrofische rechter vena spermatica niet mogelijk was, waren de concentraties van het bijnierspecifieke steroïd 21DF en van 17OHP en A in beide venae spermaticae beduidend hoger dan in het perifere bloed. Op mRNA niveau waren de bijnierspecifieke enzymen CYP11B1 en CYP11B2 en de ACTH en AII receptoren in alle tumorweefsels aanwezig. Deze bevindingen steunen de hypothese dat TART bijniereigenschappen hebben. Daarnaast is de

aanwezigheid van AII receptoren in de tumoren compatibel met de hypothese, dat ook AII een belangrijke factor voor tumorgroei is.

Alle verwijderde tumoren in onze studiegroep waren gelegen in de rete testis hetgeen kan leiden tot mechanische obstructie van de tubuli seminiferi en testiculaire beschadiging. Studies naar de kwaliteit van het residuele testisweefsel bij AGS patiënten met TART zijn nooit verricht. Daarom werden bij zeven van onze acht patiënten tijden de operatie biopten afgenomen van het omringende testisparenchym. De resultaten worden in **hoofdstuk 2.2.** beschreven. In alle biopten werd een afname van de tubulaire diameter met tubulaire fibrose gezien met in vier patiënten ook tubulaire hyalinisatie, suggestief voor irreversibele beschadiging van het testisparenchym. De kiemcellaag toonde een afname van de spermatogenese met in alle biopten een verminderde Johnson-score. Wij concludeerden dat in onze groep patiënten de aanwezigheid van TART geleid heeft tot irreversibele beschadiging van het testisparenchym.

In de literatuur zijn verschillende behandelingsmogelijkheden voor AGS patiënten met TART beschreven. Hoge doseringen glucocorticoïden kunnen leiden tot suppressie van de ACTH secretie en reductie van de tumorgrootte. In **hoofdstuk 3.1.** beschrijven wij de succesvolle medicamenteuze behandeling van een AGS patiënt met dubbelzijdige TART en azoospermie. Gedurende kortdurende behandelingen met DXM nam de grootte van de tumoren duidelijk af en trad verbetering op van de semenkwaliteit waardoor twee keer een spontane zwangerschap bij de partner van de patiënt bereikt kon worden.

Behandeling met hoge doseringen glucocorticoïden kan ernstige bijwerkingen geven en verschillende studies hebben laten zien dat deze vorm van behandeling niet altijd leidt tot een vermindering van de tumorgrootte en een verbetering van de gonadale functie. In de literatuur zijn twee AGS patiënten met TART beschreven, die succesvol door middel van een testissparende operatie behandeld werden maar het effect op de gonadale functie werd niet gerapporteerd. Daarom werden in onze groep patiënten voor en na de operatie de tumorgrootte, de symptomen van de patiënten en de gonadale functie geëvalueerd. De resultaten van dit onderzoek worden beschreven in **hoofdstuk 3.2.** In geen van onze patiënten waren na de operatie bij MRI-onderzoek resttumoren zichtbaar. Twee patiënten hadden preoperatief pijnklachten, die na de operatie verminderden. De gonadale functie verbeterde niet: semenanalyse toonde peroperatief azoospermie bij 5 patiënten en oligospermie bij een

patiënt zonder verbetering na de operatie. Alle patiënten hadden lage inhibine B spiegels zonder verbetering na de operatie. Daarom concludeerden wij, dat bij onze volwassen patiënten met langdurig bestaande TART de gonadale functie niet verbeterde ondanks verwijdering van de tumoren. Deze bevindingen worden gesteund door de histologische bevindingen, die beschreven zijn in hoofdstuk 2.2. Bij alle 8 patiënten werd voor de operatie en 6 en 22 maanden na de operatie een MRI van de testes verricht. In **hoofdstuk 4** worden de resultaten van deze radiologische evaluatie beschreven. Bij geen van de patiënten werden postoperatief nog bijnierresttumoren gevonden. Het volume van het residuele testisparenchym verminderde na de operatie significant, suggestief voor persisterende testiculaire beschadiging.

De incidentie van TART en de consequenties voor de gonadale functie op de kinderleeftijd is niet goed onderzocht. Daarom verrichtten wij bij 34 jongens met AGS, die op onze polikliniek vervolgt worden, scrotale echografie. Ter beoordeling van de gonadale functie werden bij de meeste kinderen retrospectief de LH, FSH, inhibine B en testosteron concentraties in bloed geevalueerd. De resultaten van dit onderzoek worden beschreven in **hoofdstuk 5**. De incidentie van TART in onze groep jongens bedroeg 8/34 (24%). Twee kinderen met TART waren tussen 5 en 10 jaar oud (10% in deze leeftijdscategorie) en zes kinderen waren tussen 10 en 18 jaar oud (66% in deze leeftijdscategorie). Bij twee van de acht kinderen met TART werden bilateraal tumoren gevonden. Bij alle kinderen met TART waren de LH, FSH, testosteron en inhibine B spiegels vergelijkbaar met de gemeten concentraties in de groep kinderen zonder TART, met uitzondering van twee kinderen met een geïsoleerde LH concentraties.. Alle metingen waren binnen de normale range. Hieruit kan geconcludeerd worden, dat bij jongens met AGS TART al op kinderleeftijd aanwezig kan zijn en dat in onze groep kinderen nog geen tekenen van gonadale dysfunctie aantoonbaar waren.
In tegenstelling tot het frequente voorkomen van TART bij mannen met AGS zijn ovariële bijnierresttumoren bij vrouwen met AGS zeer zeldzaam. De literatuur bevat twee case reports van histologisch gedetecteerde ovariële bijnierresten bij vrouwelijke AGS patiënten. In een eerdere studie van onze groep werden in een groep van 13 volwassen vrouwen met AGS middels MRI en echografisch onderzoek geen ovariële bijnierresttumoren gevonden. In **hoofdstuk 6** beschrijven wij een meisje met AGS, dat op jonge leeftijd overleden is aan een Addison crisis en waarbij bij post mortem onderzoek ovariële adrenale resten gevonden werden.
In **hoofdstuk 7** wordt een samenvatting en een algemene discussie van de resultaten gegeven.

8.2. Zusammenfassung

Testikuläre Nebennierenresttumore sind häufige Komplikationen bei männlichen Patienten mit Adrenogenitalem Syndrom (AGS). Diese Tumore können zur Obstruktion der Tubuli Seminiferi führen und somit die Hodenfunktion negativ beeinträchtigen. Bislang ist die Ätiologie und Pathogenese dieser Tumore nur unzureichend bekannt. In der vorliegenden Studie werden verschiedene funktionelle und klinische Aspekte der Nebennierenrestumore beschrieben. Die englische Bezeichnung für diese Tumore ist „Testicular Adrenal Rest Tumours". Aus diesem Grund werden die Tumore in dieser Dissertation kurz „TART" genannt.

Im **Kapitel 1.1.** wird eine allgemeine Einführung in das Krankheitsbild AGS gegeben. AGS ist eines der häufigsten autosomal-rezessiven Krankheitsbilder und wird durch einen Enzymmangel in der Steroidsynthese der Nebennierenrinde verursacht. In mehr als 90% wird AGS durch einen Mangel des Enzyms CYP21 (21-Hydroxylase) verursacht. Hierdurch ist die Kortisolsynthese und meist auch die Aldosteronsynthese in der Nebennierenrinde stark beeinträchtigt. Die Folge ist eine gesteigerte ACTH - Konzentration, die zur Hyperplasie der Nebennierenrinde führt (darum die englische Bezeichnung „Congenital Adrenal Hyperplasia"). Die Androgensynthese in der Nebennierenrinde hingegen ist durch den Enzymmangel nicht beeinträchtigt und aufgrund der stark erhöhten ACTH Konzentration werden vermehrt Nebennierenandrogene produziert. Diese Androgene können bereits intrauterin zur Virilisierung der äusseren weiblichen Genitalien führen. Die beeinträchtigte Kortisol- und Aldosteronsynthese führt postnatal zur Addisonschen Krise mit Dehydratation und Salzverlust. Jungen zeigen ein besonders hohes Risiko, eine Addison - Krise zu entwickeln, da Jungen in den ersten Lebenstagen keine Auffälligkeiten an den äusseren Genitalien zeigen und somit auch nicht frühzeitig diagnostiziert werden können. Darum wurde in vielen Ländern bereits das neonatale AGS-Screening eingeführt, um vor allem Jungen frühzeitig diagnostizieren und behandeln zu können.

AGS wird durch Mutationen im CYP21A2 Gen verursacht, das auf dem Chromosom 6p21.3 liegt. Dieses Gen ist zu 98% mit einem inaktiven Pseudogen (CYP21p) identisch, welches sich in unmittelbarer Umgebung des CYP21A2 Gens befindet. In dem Pseudogen befindet sich eine große Anzahl an Mutationen, die das Gen vollständig inaktivieren. Die Mutationen, die bei AGS - Patienten gefunden werden, kommen zu mehr als 60% aus dem Pseudogen, das

mittels Gen - Konversion während der Meiose auf das aktive Gen übertragen wird. Der genaue Mechanismus dieser Gen - Konversion ist nicht bekannt. In weiteren 30% führt ein ungleiches Cross-over während der Meiose zu einer kompletten Deletion des CYP21A2 - Gens. Spontane Mutationen, die nicht bei den Eltern nachweisbar sind, werden in 1 – 2% der Fälle identifiziert.

Die Symptome des AGS sind vom Ausmaß des Enzymmangels abhängig. Ein kompletter Enzymausfall führt zu schwerem Salzverlust nach der Geburt und zur Virilisierung des weiblichen Genitales (klassische Salt Wasting Form des AGS). Einige Punktmutationen führen zu einer Restaktivität des Enzyms von 1 – 2% mit genügend Aldosteronproduktion, um einen schweren Salzmangel zu verhindern. Auch bei dieser Form kommt es jedoch zu Symptomen eines Kortisoldefizits und zur Virilisierung des weiblichen Genitales (Simple Virilising Form des AGS). Andere Mutationen führen nur zu einer leichten Beeinträchtigung der Enzymaktivität mit einer Restaktivität von bis zu 30 – 50%. Diese Patienten produzieren genügend Kortisol und Aldosteron, haben jedoch ebenfalls eine leicht erhöhte adrenale Androgenproduktion, die im Kindesalter zu einer Pseudopubertas Präcox mit Wachstumsakzeleration und im Erwachsenenalter zu Menstruationsproblemen, Akne und Hirsutismus führen kann (Late onset Form des AGS).

Die Inzidenz des klassischen AGS beträgt in den Niederlanden etwa 1: 12.000. Die Inzidenz der Late onset Form des AGS ist höher und liegt bei etwa 1: 1700. Die Diagnose AGS kann durch den Nachweis der erhöhten Steroide 17-Hydroyprogesteron (17OHP) und Androstendion (A) gestellt werden bzw. durch den Nachweis von Metaboliten dieser Steroide im Sammelurin. Darüber hinaus kann die Diagnose mit Hilfe der DNA-Analyse bestätig werden. Die Behandlung besteht aus der Substitution von Glukokortikoiden, um hiermit gleichzeitig die erhöhte ACTH - Produktion in der Hypophyse zu unterdrücken, wodurch auch die Produktion von Nebennierenandrogenen abnimmt. Bei gleichzeitigem Aldosteron ist auch die Substitution von Aldosteron notwendig. Mädchen mit virilisiertem äusseren Genitale werden meist bereits im 1. Lebensjahr operativ behandelt.

Bei Eltern, die beide Träger einer Mutation im CYP21A2 Gen mit dem Risiko des klassischen AGS sind, besteht in der Schwangerschaft ein Risiko von 12.5% ein virilisiertes Mädchen zu bekommen. Diese Virilisierung kann in erheblichem Maße verhindert werden, in dem die

Mutter bereits früh in der Schwangerschaft mit Dexamethason behandelt wird, wodurch die embryonale Androgenproduktion gehemmt wird.
Erwachsene Patienten mit AGS haben häufig Fertilitätsprobleme. Im **Kapitel 1.2.** werden die häufigsten Ursachen für Infertilität bei Patienten mit AGS beschrieben.
Bei Männern mit AGS sind TART die häufigste Ursache für Infertilität. Die Inzidenz von TART liegt bei 0 – 94%, abhängig von der Patientenselektion und der Untersuchungsmethode. TART sind immer gutartig und befinden sich in der Regel innerhalb der Rete testis des Hodens. Dadurch können diese zu einer Obstruktion der Tubuli Seminiferi und auf Dauer zu einer irreversiblen Schädigung des Hodenparenchyms führen (hypergonadotroper Hypogonadismus). Eine weitere Ursache für Infertilität bei Männern mit AGS ist ein hypogonadotroper Hypogonadismus als Folge von Suppression der Hypophysen-Gonadenachse durch erhöhte Androgene bei einer nicht optimalen medikamentösen Einstellung.

Bei Frauen mit AGS kann eine Reihe von Faktoren zu einer verminderten Fertiliät führen. Erhöhte adrenale Androgene im Blut können die ovarielle Follikulogenese und die Endometriumproliferation hemmen. Weiterhin kann ein erhöhtes Progesteron, das bei nicht optimaler hormonaler Einstellung ebenfalls vermehrt durch die Nebennieren ausgeschieden wird, zur Unterentwicklung des Endometriums und zur Impermeabilität des zervikalen Mukus führen. Auch bei Frauen mit AGS können erhöhte Androgene zu einer Suppression der Hypophysen-Gonadenachse führen (hypogonadotroper Hypogonadismus).
Eine verminderte Fertilität kann bei Frauen neben den endokrinen Faktoren auch eine mechanische Ursache haben. Entweder bedingt durch die Virilisierung oder durch Operationsfolgen.

In der vorliegenden Studie werden 8 infertile Männer mit AGS und beidseitigen TART beschrieben, bei denen die Tumore mittels testessparender Operation entfernt wurden. Es werden einige funktionelle Aspekte von TART beschrieben (Kapitel 2.1.), die histologischen Befunde des umgebenden Hodenparenchyms (Kapitel 2.2.), die klinischen Resultate der operativen Behandlung (Kapitel 3.2.) und die radiologischen Befunde (Kapitel 4). Weiterhin wird ein Patient mit TART beschrieben, der erfolgreich medikamentös behandelt wurde (Kapitel 3.1.) sowie ein Mädchen, bei dem seltsame ovarielle Nebennierenreste gefunden wurden. TART sind wahrscheinlich vom Ursprung her embryonale Nebennierenzellen. Hierfür spricht unter anderem die klinische Wahrnehmung, dass hohe Dosen Glukokortikoide

die Tumorgröße beeinflussen können, sowie dass nebennierenspezifische Hormone in der Vena Spermatika von einzelnen AGS-Patienten mit TART gemessen wurden. Die Hypothese ist, dass während der embryonalen Entwicklung Nebennierenzellen aus der sich in unmittelbarer Umgebung des Hodens entwickelnde Nebennierenrinde in die Rete Testis des Hodens einnisten können und zusammen mit dem Hoden zum Skrotum deszendieren. Unter Einfluß von Wachstumsfaktoren wie ACTH können diese Zellen proliferieren und möglicherweise sogar hormonal aktiv werden. Eine nicht optimale Behandlung von AGS-Patienten mit erhöhten ACTH-Konzentrationen spielt möglicherweise eine wichtige Rolle beim Tumorwachstum. Optimalisierung der Glukokortikoid-Behandlung führte bei einigen Patienten zu einer Abnahme der Tumorgröße und zu einer Verbesserung der Hodenfunktion. Andere Fallstudien hingegen beschrieben keine Abnahme der Tumorgröße nach Intensivierung der Glukokortikoid-Therapie.

In der vorliegenden Studie wurden einige funktionelle Aspekte der TART bei acht männlichen AGS - Patienten mit beidseitigen Tumoren untersucht, die alle mittels Testes-sparender Operationen behandelt wurden. Alle Patienten waren infertil und die meisten Patienten wurden vorab erfolglos mit höheren Dosen Glukokortikoiden behandelt, um den Tumor zu reduzieren. Bei sieben dieser acht Patienten wurde während der Operation die Vena Spermatika kannuliert und Blut zur Bestimmung der nebennierenspezifischen Hormone 21-Desoxycortisol, 17OHP und A abgenommen. Zusätzlich wurden diese Hormone in simultan abgenommenen peripheren Blutproben bestimmt. Das entfernte Tumorgewebe wurde mikroskopisch untersucht. mRNA-Konzentrationen der nebennierenspezifischen Enzymen CYP11B1 und CYP11B2 sowie von ACTH und Angiotensin II Rezeptoren wurden mittels PCR-Techniken gemessen. Die Ergebnisse werden in **Kapitel 2.1.** dargestellt.
Mit Ausnahme eines Patienten, bei dem an einer Seite Kanulation der stark atrophischen Vena Spermatika nicht möglich war, waren bei allen Patienten die Konzentrationen der nebennierenspezifischen Steroide 21DF und 17OHP und A in der Vena Spermatika deutlich höher als im peripheren Blut. Auf mRNA-Niveau waren die nebennierenspezifischen Enzyme CYP11B1 und CYP11B2 sowie ACTH- und AII Rezeptoren in allen Tumoren nachzuweisen. Diese Ergebnisse bestätigen die Hypothese, dass TART Nebennierenrindeneigenschaften besitzt. Darüber hinaus kann der Nachweis von AII Rezeptoren ein Hinweis dafür sein, dass neben ACTH auch AII als möglicher Wachstumsfaktor für TART in Frage kommt. Alle entfernten Tumore befanden sich im Rete Testis mit dem Risiko der Obstruktion der Tubuli Seminiferi. Studien nach der Qualität des Hodenparenchyms bei AGS Patienten mit TART

wurden bisher nicht durchgeführt. Darum wurde bei den acht Patienten während der Operation Biopsien vom umringenden Hodenparenchym genommen und histologisch untersucht. Die Resultate dieser histologischen Studie werden im **Kapitel 2.2.** beschrieben. In allen Gewebeproben wurde eine Abnahme des tubulären Diameters mit testikulärer Fibrose gefunden. Vier Patienten zeigten darüber hinaus eine tubuläre Hyalinisation als Zeichen einer irreversiblen Schädigung des Hodenparenchyms. Weiterhin wurde eine verminderte Anzahl an Spermazellen festgestellt (verminderter Johnson Score). Hieraus ist zu schließen, dass es bei den Patienten zu einer irreversiblen Schädigung des Hodenparenchyms kam.

In der Literatur werden verschiedene Behandlungsmöglichkeiten für TART beschrieben. Zum einen kann die Behandlung mit erhöhten Dosierungen Glukokortikoiden zu einer Suppression des ACTH und damit zu einer Reduktion der Tumorgröße führen. Im **Kapitel 3.1.** wird die erfolgreiche Behandlung eines AGS-Patienten mit bilateralen Tumoren und Infertilität beschrieben, der mit jeweils kurzen Behandlungen mit Dexamethason eine deutliche Reduktion der Tumorgröße und eine Verbesserung der Hodenfunktion zeigte.

Leider kann eine Behandlung mit Dexamethason auch gravierende Nebenwirkungen haben. Darüber hinaus zeigten einige Studien, dass diese Form der Behandlung nicht immer den gewünschten Erfolg bringt. Darum wurde die Hoden-sparende Operation als mögliche Alternative eingeführt, wobei selektiv der Tumor entfernt wird und das übrige Hodengewebe erhalten bleibt. Zwei Studien beschreiben insgesamt fünf AGS Patienten mit TART, die erfolgreich mit dieser Methode operiert wurden. Der Einfluss auf die gonadale Funktion wird in beiden Studien jedoch nicht beschrieben. Darum wurden in der vorliegenden Studie sowohl vor, als auch nach der Operation die Tumorgröße, die Symptome sowie die gonadale Funktion mittels Spermaanalyse und Blutuntersuchungen evaluiert. Die Resultate dieser Untersuchungen werden im **Kapitel 3.2.** beschrieben.
Bei keinem der Patienten war postoperativ im MRT noch Tumorgewebe nachweisbar. Zwei Patienten gaben vor der Operation Schmerzen im Hodenbereich an. Nach der Operation waren sie beschwerdefrei. Die Hodenfunktion besserte sich postoperativ nicht. Spermaanalysen zeigten vor der Operation bei fünf Patienten eine Azoospermie und bei einem Patienten eine Oligospermie ohne deutliche Verbesserung nach der Operation. Alle Patienten hatten deutlich erniedrigte Inhibin B Konzentrationen, die auch nach der Operation nicht anstiegen. Auch hieraus ergibt sich, dass bei der untersuchten Patientengruppe mit lang bestehenden Tumoren das Hodenparenchym irreversibel geschädigt ist. Bei allen Patienten

wurden sowohl pre- als auch postoperativ MRT-Untersuchungen des Skrotums durchgeführt. Im **Kapitel 4** werden die Resultate dieser radiologischen Evaluation beschrieben. Bei keinem Patienten konnte postoperativ Tumorgewebe nachgewiesen werden. Das verbliebene Hodengewebe nahm postoperativ deutlich ab, suggestiv für eine persistierende oder auch additionale Schädigung des Hodens durch die Operation.
Die Inzidenz der TART und die Konsequenzen im Kindesalter sind bislang nicht bekannt. Darum wurde bei 34 Jungen mit AGS, die in unserer Klinik behandelt werden, skrotale Ultraschalluntersuchungen durchgeführt. Weiterhin wurden die LH-, FSH-, Inhibin B- und Testosteronkonzentrationen im Blut bestimmt, um die Hodenfunktion zu evaluieren. Die Resultate dieser Untersuchung werden im **Kapitel 5** beschrieben. Die Inzidenz der TART in unserer Studiengruppe betrug 8/34 Kinder (24%). Zwei Kinder waren im Alter zwischen 5 – 10 Jahre, sechs Kinder waren zwischen 10 – 18 Jahren alt. Alle Kinder mit TART zeigten LH-, FSH-, Inhibin B- und Testosteronspiegel, die vergleichbar waren mit denen von Kindern ohne TART. Alle Messungen lagen innerhalb des Normbereichs für das entsprechende Pubertätsstadium mit Ausnahme von 2 Kindern mit erhöhten LH Werten.. Hieraus ergibt sich, dass TART bereits im Kindesalter nachweisbar sind und dass in dieser Studiengruppe keine Schädigung des Hodens nachweisbar ist.

Im Gegensatz zu männlichen Patienten mit TART sind ovarielle Nebennierenreste bei Frauen mit AGS sehr selten. In der Literatur werden zwei Fälle bei weiblichen AGS Patientinnen mit histologisch nachweisbaren ovariellen Nebennierenresttumoren beschrieben. In einer früheren Studie unserer Fachgruppe wurden bei 13 Frauen mit AGS mittels MRT und Ultraschall keine overiellen Nebennierenresttumoren gefunden. In **Kapitel 6** wird ein Mädchen mit AGS beschrieben, das im ersten Lebensjahr an einer Addisonschen Krise verstorben ist. Bei der Obduktion wurden ovarielle Nebennierenreste gefunden.

Im **Kapitel 7** wird eine Zusammenfassung der Studie sowie eine allgemeine Diskussion der Ergebnisse gegeben.

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Karel de Weert: Beste *Karel*, wij hebben een geweldige studietijd gehad en alles samen doorlopen. Ik ben erg blij dat jij me ook nu als paranimf weer bij wilt staan. Helaas hebben wij in de laatste jaren door alle drukte weinig tijd gehad om elkaar te ontmoeten. Ik hoop dat hier in de komende jaren weer verandering in komt.

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Curriculum vitae

Hedi Leonie Claahsen – van der Grinten was born on May 17th 1966 in Kranenburg, Germany. She attended High School in Kleve, Germany, from which she graduated with "A" levels in 1985 (Abitur). Thereafter, she started her three years training as a children's nurse at the St. Antonius Hospital in Kleve, Germany (Head: Dr. H. Schumacher) finishing it in October 1988 with a diploma (Staatsexamen Kinderkrankenpflege). From 1988 until 1989 she attended a course in physics and chemistry as a preparation for the Medical Study in the Netherlands.

In 1989 she started her Medical Study at the Radboud University Nijmegen, The Netherlands. She passed her doctoral exam in 1993 (cum laude). During this time she worked as an assistant at the department of anatomy and took part in a research project at the centra animal laboratory. In addition, she worked as a children's nurse in the St. Antonius Hospital in Kleve, Germany and taught anatomy and special children's care in the Children's Nurse School in Kleve, Germany.

In 1993 she started her medical practical training finishing it with a medical degree in March 1996. From March 1996 until November 2002 she completed her paediatric training at the Radboud University Nijmegen Medical Centre, The Netherlands (Head: Prof. R.C.A. Sengers). In 2002 she started her training in Paediatric Endocrinology at the Department of Paediatric Endocrinology of the Radboud University Nijmegen Medical Centre, The Netherlands (supervisors: Dr. B.J. Otten and Dr. C. Noordam). She was registered as Paediatric Endocrinologist in January 2006. During this time she started her studies on patients with congenital adrenal hyperplasia, which are subject of this thesis. At present she works as a staff member at the Department of Paediatric Endocrinology in the Radboud University Nijmegen Medical Centre, The Netherlands. She is married to Lûi Claahsen. They have got 3 children: Nina (1999), Tobias (2000) and Lukas (2002).

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